ISSN No.: 0973-8045

THE PHARMACIST

The Pharmacist

Formulation

Biopharmaceutics

Pharmacokinetics

Pharmacology

Medicinal Chemistry

Analytical Chemistry

Herbal and Traditional Medicine

Biotechnology

VOLUME 2 NUMBER 2 2007

THE PHARMACIST

An International Bi-annual Journal.

Editor

Dr.A.Balasubramaniam. M.Pharm., PhD., Director, TIT- Pharmacy, Bhopal

Associate Editor

Dr. B. K. Dubey M.Pharm., PhD., Director ,TIT College of Pharmacy, Bhopal
Dr. B. Sangameswaran., M.Pharm., Ph.D., Vinayaka Mission University, Salem.
Dr. Vimal Kumar M.Pharm., PhD., Director PG Studies, TIT Pharmacy, Bhopal
Mr. N. Balakrishnan M. Pharm., TIT Pharmacy, Bhopal
Mrs.B.Thirumagal MSc., M.R. Labs., Bhopal
Mr. C.S. Senthil Kumar. MSc., M.Phil., Biotech., M.R. Labs., Bhopal

Advisory Board

Dr. Katsumi Kobayashi, Shizoka, Japan
Dr. K. Sadasivan Pillai, Orchid Research laboratories Ltd., Chennai
Dr. Ramesh Narayanan, GTX Pharamceuticals, Memphis, U.S.A.
Dr. O.P.Katare, Punjab University, Chandigarh
Dr. Anupam Pathak M.Pharm., Ph.D., HOD Pharmacy, Barkatullah University, Bhopal
Dr. B.Jayakar M.Pharm., Ph.D., HOD Pharmacy, Vinayaka Mission University, Salem
Dr. Satish Nayak M.Pharm., PhD., Director BITS- Pharmacy, Bhopal

Information

The Pharmacist is a bi-annual journal. Inland Institutional subscription for one year is Rs. 1000/-, while individual subscription is Rs. 600/-. Foreign institutional subscription is $40 while foreign individual subscription is $30. Payment in respect to Subscription, Advertisement, Reprints may be sent by cheque, bank draft marked payable to "The Pharmacist". In case of outstation cheques, add Rs 30/- as bank collection charges. For further particulars and advertisement rates etc. please contact the Editor, The Pharmacist, A-14 Siddharth Lake City, Anand Nagar, Bhopal 462021.
E-mail: thepharmacist_ijp@rediffmail.com.

Note: The editor does not claim any responsibility, liability for statements made and opinions expressed by the publishing authors

Editorial

At the outset, I would wish to greet all the contributors, readers and the scientific community as a whole – a happy and prosperous new year.

I would also like to thank you for your warm reception to "The Pharmacist" by way of manuscript submission and subscription. I once again solicit your support in further enhancing the readership base by recommending "The Pharmacist to institutions and individuals at every opportunity.

Suggestions and criticism are gladly welcomed from well meaning readers, whose clear intentions are to enhance the quality of "The Pharmacist".

At this juncture I would also like to acknowledge Mr. Anand Jain of J.K. Publishers for his unrelenting and selfless support regardless of his busy schedule without which this journal would not have been as it is today.

Dr. A. Balasubramaniam
The Editor

This One
9L69-802-W5R4

The Pharmacist 2 (2) 1-5 (2007)

Study of Hepatoprotective Activity of *Portulaca oleracea* L. on Rifampicin Induced Hepatotoxicity in Rat

**A.S. Kulkarni*, B.D. Siraskar, A.D. Deshpande, A.V. Kulkarni,
S.M. Dhonde, and Bingi**
Department of Pharmacology , Padam. Dr. D.Y. Patil Institute of Pharmaceutical Science and Research, Pimpri, Pune 411018, India.

ABSTRACT

Evaluation of hepatoprotective activity against Rifampicin induced hepatotoxicities in rat, of hydroalcoholic extract of the stems and leaves of Portulaca oleracea L. was carried out. The hydroalcoholic extract of Portulaca oleracea L. exhibited significant hepatoprotective activity ($P < 0.01$) close to silymarin, a well known as hepatoprotective agent.

Key words: *Portulaca oleracea L., Rifampicin, hepatotoxicity.*

INTRODUCTION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairments of its function may lead to many implications on one's health. Management of liver diseases is still a challenge to a modern medicine. The modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representatives are of phytoconstituents.

The encouraging results of the studies on the stems and leaves of Portulaca *oleracea* L. (Ghole, fam. Portulaceae) on preliminary basis for their hepatoprotective activity against a classical chemical (carbon tetrachloride) induced hepatotoxicity initiated us to assess their hepatoprotective activity against drug induced hepatotoxicity.

Portulaca oleracea has been used as folk medicine in different countries to treat different ailments in humans. In the Indian subcontinent, the plant is reputed to reduce ulcers, tumors, inflammation and good for asthma, leprosy and piles. It is regarded as cooling diuretic, refrigerant, and alterative, used in liver diseases, pulmonary diseases and as a tonic. It is also used as a dietary supplement in scurvy[1,2]. In china it is used in the treatment of atheroma and as an antifungal and antiviral agent. It also decreases blood sugar, enhances immunity, and adjusts blood lipids[3] .

A review of records in scientific literature indicated that *Portulaca oleracea* has many medicinal uses. Reported pharmacological studies on *Portulaca oleracea* include antibacterial[4], analgesic and anti-inflammatory[5], relaxant effect on skeletal and smooth muscle[6], neuropharmacological activity[7], wound healing activity[8]. Bronchodilatory effect in asthmatic patients[9] antioxidant activity[10], antiulcer activity[11], and antitussuve effect[12].

Chemically, *Portulaca oleracea* contains many biologically active compounds, some of them being nutritive. Some of the biologically active compounds include alkaloids[13] omega-3 fatty acids, coumarins, tannins, flavonoids, cardiac glycosides . The Hepatoprotective activity of *Portulaca oleracea* may be mediated by its antioxidant agents such as Vitamin E, Vitamin C, glutathione, β-carotene and β-sitosterol. Acute toxicity study was carried out on *mice* were on 2000mg/kg *Portulaca oleracea* was found to be safe and 1/10th of that dose converted for *rat* (70, 140, 280mg/kg) and was taken for further study.

MATERIALS AND METHODS

Plant material

The fresh whole plants of *Portulaca oleracea* L. were collected during the winter from a farm in Pune, India. The plant specimen was authenticated by B.S.I. Pune, India, (voucher specimen no. AVK 11).

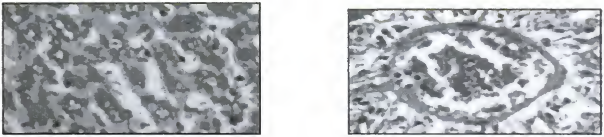
Preparation of the hydroalcoholic extract of *portulaca oleracea* linn.

The dried leaves were extracted by cold maceration using 10% v/v aqueous ethanol in 1:8 ratios for 72hr., at room temperature. The powder residue was removed by filtration. The solvent was then evaporated, under reduced pressure, in a rotary evaporator at 42-45°C.

Experimental animal

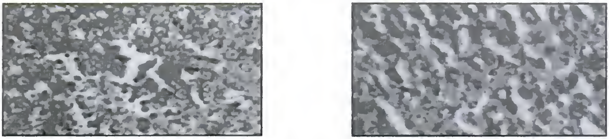
All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA Reg. No.198/99) and were approved by the Institutional Animal Ethics Committee. *Wistar rat* (*Rattus norvegicus*) weighing between 180 to 200 gm of either sex was used. The above animals of either sex were purchased from National Toxicology Center, Pune. Animals had free access to standard pellet diet and water *ad libitum*.

**Corresponding author - E-mail: ahijinkya67@yahoo.com*



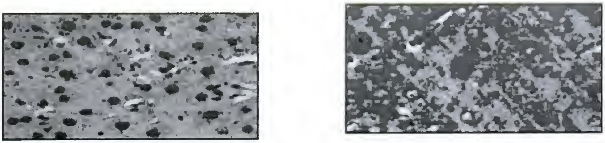
Photomicrograph of rat liver of control group showing normal liver. HxEx400

Photomicrograph of rat liver of Negative control group showing portal infiltration along with extensive degeneration and necrosis of the hepatocytes HxEx400



Photomicrograph of rat liver of silymarin treated group showing no evidence of necrosis and or necrotic infiltrate. HxEx400.

Photomicrograph of rat liver of PO 70 treated group showing few regeneration changes with areas of necrosis and inflammatory infiltrate. HxEx400.



Photomicrograph of rat liver of PO140 treated group showing no evidence of necrosis and close to normal. HxEx400.

Photomicrograph of rat liver of PO 280 treated group showing no evidence of necrosis and close to normal. HxEx400.

Fig.1 Effect of PO on histopathology of rat liver in rifampicin induced hepatotoxicity.

Table 1
Effect of hydroalcoholic extract of *Portulaca oleracea* treatment on different biochemical parameters in Rifampicin induced hepatotoxicity.

Serum Biochemical parameters	Groups					
	Normal Control	Negative control	Positive control	PO-70	PO-140	PO-280
AST (U/ml)	46.58 ± 2.44	122.26 $^{\#\#}$± 4.89	58.49 ± 2.85	114.17 ± 4.49	104.12 ± 3.26	63.21 ± 3.06
ALT (U/ml)	40.25 ± 2.24	95.84 $^{\#\#}$ ± 5.30	56.21± 2.04	82.54* ± 3.67	74.58 ± 3.47	69.54 ± 2.85
ALP (KA units/dl)	13.54 ± 1.22	52.49 $^{\#\#}$ ± 2.24	17.87± 1.67	48.52 ± 2.04	36.89 ± 1.55	23.54 ± 1.14
TB (mg/dl)	0.58 ± 0.020	1.56 $^{\#\#}$ ± 0.061	0.86 ± 0.032	1.36 ± 0.057	1.02 ± 0.048	0.94 ± 0.036
TP (gm/dl)	10.14 ± 0.83	5.47 $^{\#\#}$ ± 0.41	9.08 ± 0.67	5.01 ± 0.53	8.01 ± 0.39	8.82 ± 0.40

n=6. Values are expressed as Mean ± S.E.M.**=$p < 0.01$ when compared with Negative control $^{\#\#}$ = $p < 0.01$, when compared with Normal control Statistically analyzed by One Way ANOVA followed by Dunnett test.
PO- *Portulaca oleracea*

Treatment Schedule For Rifampicin Induced Hepatotoxicity[14]

Group I: Normal control Group- The animals in this group received distilled water (1ml/100gm, per oral) as vehicle in 4 divided doses. The biochemical estimations were done on after 48 hr. after overnight fasting of animals.

Group II: Negative Control Group- The animals in this group received rifampicin (1gm/kg, per oral in 5% gum acacia) at day one. The biochemical estimations were done after 48 hr. after overnight fasting of animals.

Group III: Positive control Group- The animals in this group received rifampicin (1gm/kg, per oral in 5% gum acacia) at day one after the 30 min. Silymarin (100 mg/kg, per oral) was administered in four divided dose. The biochemical estimations were done after 48 hr. after overnight fasting of animals.

Group IV: Hydroalcoholic extract of (*Portulaca oleracea* -70) - The animals in this group received rifampicin (1gm/kg, per oral in 5% gum acacia) at day 1st after the 30 min. hydroalcoholic extract of *Portulaca oleracea* (70mg/kg, per oral) was administered in four divided dose. The biochemical estimations were done after 48 hr. after overnight fasting of animals.

Group V: Hydroalcoholic extract of (*Portulaca oleracea* -140) - The animals in this group received rifampicin (1gm/kg, per oral in 5% gum acacia) at day 1st after the 30 min. hydroalcoholic extract of *Portulaca oleracea* (140mg/kg, p.o.) was administered in four divided dose. The biochemical estimations were done after 48 hr. after overnight fasting of animals.

Group VI: Hydroalcoholic extract of (*Portulaca oleracea* -280) - The animals in this group received rifampicin (1gm/kg, per oral in 5% gum acacia) at day 1st. after the 30 min. hydroalcoholic extract of *Portulaca oleracea* (280mg/kg, per oral) was administered in four divided dose. The biochemical estimations were done after 48 hr. after overnight fasting of animals.

Biochemical estimations

On 2nd day after over night fasting of animals all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 7000 rpm for 15 min. at 5° C. The separated serum was used for estimation of AST, ALT, ALP, TP and TB.

Histopathological investigations

On 2nd day the animals were sacrificed and abdomen was cut open, the liver was dissected out and stored in 10% formalin solution. The liver was processed for the histopathological investigations.

Statistical analysis:

The results are expressed as mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Dunnett test. Value of p less than 5% (i.e. $p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION :

Rifampicin, a broad spectrum antitubercular drugs, is hepatotoxic on chronic usage. It is metabolised to an active metabolite, 25- desacetyl rifampicin. It reduces drug metabolising enzymes and actively and specifically binds to RNA polymeases and there by inhibits the synthesis of all forms of RNA Thus, by inhibiting nucleic acid and protein synthesis it induces fatty liver and finally cirrhosis. The rats intoxicated with rifampicin elevated significantly ($p<0.01$) the serum biochemical parameters[15].

In the present study, the AST, ALT level increased by rifampicin treatment was significantly ($p<0.01$) reduced by hydroalcoholic extract of *Portulaca oleracea* (140 and 280 mg/kg) after 48 hr.

ALP activity is related to functioning of the hepatocyte cells. This ALP level was reduced significantly by hydroalcoholic extract of *Portulaca oleracea* (140 and 280 mg/kg) ($p<0.01$) after 48 hr.

The total bilirubin and total protein level was also restored to the normal level by the hydroalcoholic extract of *Portulaca oleracea* (140 and 280 mg/kg) ($p<0.01$) after 48 hr.

The serum level of biomarkers in *Portulaca oleracea* (140 and 280 mg/kg) treated group was restored to normal level. The result indicates that treatment with hydroalcoholic extract of *Portulaca oleracea* (140 and 280 mg/kg) protects liver against rifampicin induced hepatotoxicity.

The histopathology of the liver gives the evidence for the protection imparted by the hydroalcoholic extract and Silymarin. Control group shows normal hepatic cells. Where as administration of rifampicin in negative control group showed portal infiltration along with extensive degeneration and necrosis. Treatment with Silymarin showed normal histological appearance with no sign of necrosis. Where as *Portulaca oleracea* 140 and 280 treated group showed close to normal. The overall structure was normal.

The Hepatoprotective activity of *Portulaca oleracea* may be mediated by its antioxidants that it contains such as Vitamin E, Vitamin C, Flavonoids, β-carotene, β-sitosterol and omega- 3 fatty acid.

CONCLUSION

Taking into consideration the results obtained in the present investigation, it can be concluded that hydroalcoholic extract has a definite hepatoprotective and regenerative activity; hence it could be use in the treatment of liver disorders like liver dysfunction, viral hepatitis and various alcoholic liver disorders.

REFERENCES

1 Nadkarni K.M., Indian Materia Medica,. 3rd ed. Mumbai: Popular Prakashan; 2000. p.1005-1006

2 Rasheed A.N., Afifi F.U., Disi A.M., Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. in *Mus musculus* JVI-1, Journal of Ethnopharmacology, 2003; 88: 131-136.

3 Sun J., Guo B., Zang H., Zang J., Zang T., Chemical composition from *Portulaca oleracea* L., Journal of. Chinese Pharmaceutical Sciences, 2004; 4: 150-157.

4 Zhang, X. J., Ji. Y. B., Qu, Zh. Y., Xia, J. Ch., Wang, L., Experimental studies on antibiotic functions of *Portulaca oleracea* L. in vitro, Chinese J Microecol. 2002; 14(5): 277-280.

5 Chan, K., Islam, M. W., Kamil, M., Radhakrishnan, R.,Zakaria, M. N. M.Habibullah, M.and Attas, A., The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. Sativa (Haw.) Celak. J. Ethnopharmacol., 2000, (73): 445-451.

6 Okwuasaba F., Ejike C., Parry O., Effect of an aqueous extract of *Portulaca Oleracea* Linn. leaves on smooth muscle and rat blood pressure, Journal of. Ethnopharmacology, 1988; 22: 33-44.

7 Radhakrishnan R., Attas A., Chan K., Chen H.B., Islam M.W., Zakaria MNM, Neuropharmacological actions of *Portulaca oleracea* L. subsp. Sativa (Hawk.),Journal of Ethnopharmacology, 76; 2001: 171-176.

8 Rasheed, A. N., Afifi, F. U. and Disi, A. M., Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. in *Mus musculus* JVI-1. J. Ethnopharmacology., 2003, 88: 131-136.

9 Malek F., Boroushaki M.T., Boskabady M.H., Tohidi M., Bronchodilatory effect of *Portulaca oleracea* L. in airways of asthmatic patients, Journal of Ethnopharmacology., 2004, 93: 57-62.

10 Towatana N.H., Chaiyamutti P., Mahabusarakam W., Panthong K. and Rukachaisirikul V., Antioxidative and free radical scavenging activities of some plants used in Thai folk medicine, Pharmaceutical Biology, 2006, 44(3): 221-228.

11 Karimi G., Ettehad N., Hosseinzadeh H., Evaluation of the gastric antiulcerogenic effect of *Portulaca oleracea* L. extracts in mice, Phytotherpy Reserch., 2004, 18(6): 484-487.

12 Boroushaki M.T., Boskabady M.H., Malek F, Antitussive effect of *Portulaca oleracea* L. in guinea pigs, Iranian Journal of Pharmaceutical Research, 2004; 3: 187-190.

13 Xiang L., Ding Y, Du L, Wang R, Wang W. and Xing D., Alkaloids from *Portulaca oleracea* L., Journal of Ethnopharmacology, 2005, 66: 57-62.

14 Bafna A.R., Mishra S.H., Effect of methanolic extract of Achyranthes aspera Linn. on rifampicin induced hepatotoxicity in rats, Ars. Pharm. 2004, 45; (4): 343-351.

15 Bowman W.C., Rand M.J., Textbook of pharmacology, Blackwell scientificpublication, Oxford London, 1982; 2nd ed. p. 34-39.

The Pharmacist 2 (2) 7-10 (2007)

In vitro Screening of *Ziziphus jujuba* for their Antioxidant and Anti-adipogenic activity

A.R.Srividya* P. Hari Balaji, H. Raghu and P. Vijayan
Department of Pharmaceutical Biotechnology, J.S.S. College of Pharmacy, Ooty – 643 001

ABSTRACT

In the present work, various studies such as antioxidant, cytotoxic, antimicrobial, activity of leaves of Ziziphus jujuba has been carried out. Ziziphus jujuba leaf extract exhibited potent antioxidant activity with IC$_{50}$ *value of 2.51 ± 0.503 ¼g/ml. Ethanolic extract of Ziziphus jujuba leaf showed good cytotoxic activity against normal, cancer and adipocyte cell line. This leaf extract showed moderate inhibitory activity against the selected microorganisms. The MIC values were found to be between 500 – 1000 ¼g ml.*

Key words: *Antioxidant, antimicrobial, anti - adipogenic, Cytotoxicity.*

INTRODUCTION

Zizipus jujuba plant was collected from the road sides in Erode in the month of May 2005. It was authenticated by Dr. S. Rajan, Botanical survey of India, Medicinal plants survey and collection unit, Government Arts College, Ootacamund, Tamilnadu, India. *Ziziphus jujube* belongs to the family Rhamnaceae.[1,2] It is cultivated in dry lands in India, srilanka, Afghanistan, China, Australia and Africa. Traditionally root is used to treat headache biliousness. The bark is used to treat boils, ulcers, dysentery and diarrhea. The bark is also used as astringent in gingivitis. The leaves are used to treat biliousness, diarrhea and antipyretic, reduces obesity, scabies and other skin diseases. The smoke from the dried leaves is inhaled for the treatment of cold and cough. The ripe fruit is cooling, indigestible, aphrodisiac, tonic laxative, invigorating and remove biliousness having sensation, thirst vomiting , good in constipation and blood diseases. The dry fruit are used as laxative and appetizers, remove impurities from the blood and allays thirst. The seeds are acrid and sweetish, tonic, aphrodisiac, cure eye disease, cough asthma, thirst "vata" burning [1,2]

This plant contains fragufoline alkaloid: Mauritines A-H. saponnins jujuboside A,B; alkaloids nummulurine D-H, K; amphibines B,D and F hyodricanine A jubanine A jubanine B: yuziphines. Bark and root contains triterpenoid zizyberanelic acid. Bark contain alkaloids-Mauritines A-F; amphibines B-D and F, Frangufolin. Stem contain sapogenin, zizogenin. Leaves contain Rutin, demmarane, saponin, flavanoids- ziziphin

Jujubesaponins I- III. Jujubogenin glycosided. A fruit contain stephanine, N- normuciferine, asimiboline, cyclopeptide alkaloids, frangufoline alkaloids – jujunins A and jujunins B, vitamin C. Seeds contain spinosin, swertisin, sinapolyspinosin, p- coumaloylspinosin, glycosylisoswertisin, vicenin -2 flavanoid- apigenin [3,4].

MATERIALS AND METHODS

The collected leaves were separated from the rest part of the plant and shade dried. Then the dried leaves were coarsely powdered. The powdered leaves materials were extracted separately using 50% ethanol by cold maceration method. The extracts were concentrated to dryness under reduced pressure and the controlled temperature [5] The results were tabulated in Table – 1. For this leaf extract phytochemical constituents such as total phenolic content [6], total flavonol content [6,7] were estimated. Antioxidant studies were also performed by DPPH method [8], Nitric oxidc radical inhibition activity method [9], Hydroxyl radical scavenging activity by PNDA (p- nitrosodimethyl aniline) method [10], Scavenging Hydrogen peroxide radical [11] ABTS radical cation scavenging method [12] and scavenging of super oxide radical by alkaline DMSO method [13].Ethanolic extract of the leaf were also screened for their Cytotoxicity by MTT Assay method [14] and sulphordamine B (SRB) Method [15]. The extracts of the leaves were screened for their antimicrobial activity against the selected microorganism by two fold dilution method to determine the minimum inhibitory concentration [16]. Extracts were tested for their anti adipogenic activity in 373- L1 cell line. The concentration of the extract chosen for the testing were below their CTC$_{50}$ value and two dilution. Adipogenesis was induced by IBMX (3- iso butyl 1- Methyl xanthane), dexamethazone and insulin. The fat formation was estimated using oil red O staining. The absorbance was measured at 490 nm [17,18].

**Corresponding author - E-mail: pharmarsrividya@yahoo.com*

Table 1
Percentage of Yield of The Ethanolic Extract of *Ziziphus Jujuba*

Name of the plant	Part of the plant	weight of the Sample (g)	weight of the crude extract Obtained (g)	Percentage of yield
Ziziphus jujuba	leaves	225	32.95	14.6%

Table 2
Phyto-Chemical Constituents of *Ziziphus Jujuba* Leaves

Test	Ziziphus jujuba Leaves
Glycosides	+
Tannins	-
Saponines	+
Flavonoids	+
Steroids	-
Terpenoids	-
Carbohydrates	-
Protein	-
Total Phenolic content in Percentage	51.35
Total flavonol content mg/g	7.7

Table 3
Antioxidant Activity by Various Methods for *Ziziphus Jujuba*

Methods	IC $_{50}$ µg/ml For the Ziziphus jujube leaves
DPPH method:	
Standard:	30.5 ± 5.26
Ascorbic acid	2.69 ± 0.05
Rutin	3.91 ± 0.1
Nitric oxide Method	
Standard:	365 ±10.52
Rutin	68.44 ± 2.56
PNDA method	
Standard:	>1000
Ascorbic acid	>1000
Rutin	>1000
Hydrogen peroxide method	
Standard:	>1000
Ascorbic acid	187.33 ± 3.93
Rutin	36.16 ± 0.16
ABTS method	
Standard:	2.51 ± 0.503
Ascorbic acid	11.25 ± 0.49
Rutin	0.51 ± 0.01
Alkaline DMSO Method	
Standard:	>1000
Ascorbic acid	> 1000
Rutin	> 1000
Total antioxidant activity	
Phosphomolybdenum Method.	0.96 ± 0.031

Table 4
Results for the Cytotoxic Studies for *Ziziphus Jujuba* Leaves.

Cell type	cell line used	MTT assay µg/ml	SRB assay µg/ml
		Leaves	Leaves
Normal	Vero	250	177.5
Cancer	L-929	93.75	160
Adipocyte	373 – LI	105	97.5

Table 5
Results for the Anti Microbial Activity of *Ziziphus Jujuba* Leaves.

Test organisms	Minimum Inhibitory concentration in µg/ml
	Leaves
Gram Positive:	
Bacillus subtilis	500
Staphylococcus aureus	500
Gram Negative:	
Pseudomonos aeruginosa	500
Klebselia pneumonia	500
Mycobacterium pheli	1000
Fungi:	
Candida albicans	500
Aspergillus niger	500

Table 6
Results for Adipogenic Activity of *Ziziphus Jujuba.*

Extract	concentration in µg/ml	Absorbance at 490 nm
Leaves	150	0.424
	100	0.682
Cell control		0.207
Adipogenesis control		0.945

RESULTS AND DISCUSSIONS

The leaf of the *Ziziphus jujube* gave 14.6 % percentage yield of the extract. Table -1. Total phenolic content in the leaf was found to be 51.53. Table – 2. *Ziziphus jujube* leaves extract showed potent anti-oxidant activity with IC$_{50}$ value of 2.51 ± 0.503 ¼g/ml . The ethanolic extract of leaf was screened for their Cytotoxicity by MTT assay method. The extracts showed CTC$_{50}$ values ranging from 125 – 250 ¼g/ml against normal, vero cell lines. The methanolic extract of the leaves showed good cytotoxic against normal, cancer and adipocyte cell lines by the SRB assay method. The extracts showed CTC$_{50}$ values ranges from 103 – 400 ¼g/ml. The extracts showed inhibitory activity against all the selected tested microorganism. The MIC was found to be 500 - 1000 ¼g/ml.

SUMMARY AND CONCLUSION

The ethanolic extract showed potent antioxidant activity by ABTS method when compared to the standard. The leaf extract found to possess cytotoxic activity in the concentrations of 125 – 250 ¼g/ml. it also found to possess potent Adipogenic effect and also anti-bacterial activity against all the selected microorganism. It is worth to continue this project for isolations of the individual constituents in pure form, for its potent anti-oxidant, anti-adipogenic and antimicrobial and cytotoxic property.

REFERENCES

1. Kiritkar K.R., Basu B.D. Indian medicinal plants. 2nd Ed. Published by Lalit Mohan Basu: 1984: p- 1439.
2. Yoganarashimha S.N. Medicinal plants of India – Tamilnadu. Vol II: 2000: p-603.
3. Moro C.O. Basile G. Obesity and medicinal plants. Fitoterapia; 2000: 71: p- 73-82.
4. Puricelli L. Dell Aica. Sartar L. Preliminary evaluation of inhibition of Matrix – Metallo protease MMp-2 and MMp-9 by Passiflora edulis and Passiflora foetidia aqueous extracts. Fitoterapia: 2003: 74: (3): p- 302.
5. Kumari S.C. Govindasamy S. Sukumar E. Lipid lowering activity of Eclipta prostrate in experimental hyperlipidemia. J Ethano Pharmaco: 2006: 105: p-332.
6. Mills S. Bone K. Modern Herbal Medicine. Principles and practice of phytotherapy. Churchill Livingston; Harcount publishers 2000: p- 32.
7. Brielmamm H.L. Kaufman P.B. Sarawarber Duke J.A. Natural products from plants 1999: 1: p- 22.
8. Sanchew. Moreno C. Methods used for evaluating the free radical scavenging activity in food and biological systems. Food science and Technology: 2002: 8: (3): p-121.
9. Elizabeth Kunchandy, Rao M.N.A. Effect of curcumin on hydroxyl radical generation through Fenton reactions. Int J of Pharmaceutics; 1989: 58: p-173.
10. Kellogg E.W. Fridovich I. Liposome oxidation and Erythrocytes lysis by Enzymatically generated super oxide and Hydrogen peroxide. J of Biological chem.; 1997: 252: p- 6721.
11. Robert R.E., Nicoletta Pellegrini, Ananth pannala, Free radical Biology and Medicine 1999; 26: p-1231-1234.
12. Gudda Darangavanahally K. Jayapraksasha. Antioxidant activities of flavidin in different in-vitro model systems. J of Bioorganic and Medicinal Chemistry; 2005: 12: p- 141.
13. Pongtip Sithisarn.Roongtawan supabphol. Antioxidant activity of Siamese Neem tree. J of Ethano pharmacology; 2005: 99:p- 109.
14. Jaffery M.E. Linda S. Armstrong. Andrew O.M.A. . Rapid and simple MTT- based spectrophotometeric assay for determining drug sensitivity in monolayer culture. Tissue culture methods; 1988:11: p-15.
15. Philip S. Rista S. Dominic S. Anne M. New colorimetric Cytotoxicity Assay for Anticancer drug screening. J of National cancer Institute;1990: 82: p-1107.
16. Michael J Pelzer. Chan E.C.S. Microbiology, 4th Ed. 1988: p- 535.
17. Anne W Harmon . Joyce Herp. Differential effect of flavonoids on 3T3-L1 Adipogenesis and lipolysis. American J of Physiology and cell physiology; 2001; 280: 4: p- 807.
18. David M Barnes. Paul R. Effect of inorganic HgCl$_2$ on Adipogenesis. In vitro toxicology and Alternative sciences; 2003: 7: p- 47-55.

The Pharmacist 2 (2) 11-22 (2007)

Evaluation of Anti-Inflammatory Potential of *Pisonia grandis* Leaf Extract in Rats.

N.Balakrishnan[1]*, A.B.Panda[2], Sanjiv Singh[2], Navin R Raj[2], C.S.SenthilKumar[3] and A.Sathish[3]
[1] Faculty of Pharmacy, Vinayaka mission's University, Salem-636008, Tamilnadu.
[2]Technocrats Institute of Technology – Pharmacy, Bhopal - 462021(M.P).
[3]NRI Institute of Pharmacy, Bhopal (M.P).

ABSTRACT

The methanol extract of leaf of Pisonia grandis was evaluated for anti-inflammatory activity in rats at a dose level of 200,300 and 500 mg kg in carrageenin induced paw oedema and cotton pellet methods. The methanol extracts of Pisonia grandis leaf exhibited significant anti-inflammatory activity comparable to standard drug phenylbutazone.

Key words: *Pisonia grandis, anti-inflammatory, methanol extract.*

INTRODUCTION

Pisonia grandis (Nyctaginaceae) is an evergreen tree, 9-12m height and widely distributed through out India. It's mainly cultivated as an ornamental plant in gardens and has been used for various disorders in traditional system of medicine. Leaves are 15-25 cm long; ovate-oblong, pale greenish colour[1].The leaves of the plant are used in treatment of fever, inflammation and ulcer[2, 3]. The root of this plant is used as purgative[3]. The present study was undertaken to evaluate the anti-inflammatory activity of methanol extract of *Pisonia grandis* leaf.

MATERIALS AND METHODS

Plant Material

The leaves of *Pisonia grandis* were collected from various parts of Chennai, Tamilnadu, during the month of September .The plant was taxonomically identified by Dr.P.Brinda, Botanist, Captain Srinivasamoorthi Drug Research, Chennai. The Voucher specimen (VMCOP/Pg/01) is preserved in Department of Pharmacognosy, Faculty of Pharmacy, Vinayaka mission's University, Salem for further reference.

Extraction procedure

The powdered plant material was extracted with methanol using soxhlet apparatus and the solvent was completely removed under reduced pressure using rotary flash evaporator. The yield of semisolid mass (extract) was 7.2 % w / w. The extract was suspended in 5% w / w carboxymethyl cellulose (CMC) for anti-inflammatory activity.

Toxicity studies

Wister albino mice (18-25 gm) of either sex were used in acute toxicity studies. Toxicity and gross behavioral studies[4] of methanol extract were carried out in oral doses of 100 to 2000 mg / kg body weight using albino mice. After test extracts administration, animals were observed during first 2hr. for gross behavioral changes and once in 30 min. for next 4hrs. and subsequently the animals were observed once in 24hrs. for next 72hrs. to find out percentage mortality.

Phytochemical Analysis

In preliminary phytochemical analysis[5] the methanol extract of leaf of *Pisonia grandis* showed positive response for alkaloids, flavonoids, tannins, steroids, resins and triterpenoids.

Evaluation of Anti-inflammatory Activity

Carrageenin –Induced Rat Paw Oedema

The rats were divided into five groups of six animals in each. Acute inflammation was induced by the sub-planter administration of 0.1ml of 1% carrageenin in normal saline in the right hind paw of the rats. The paw volume was measured at 0hr.and 4hrs. after carrageenin injection, using plethysmometer[6].The first group received normal saline (3 ml / kg) and second group received phenylbutazone(100 mg / kg) orally. Third, fourth and fifth groups were treated with the methanol extracts 200,300 & 500 mg / kg orally respectively. The animals were pretreated with the drug 1hr. before the administration of carrageenin.

Cotton Pellet-Induced Granuloma

The rats were divided into five groups of six animals in each. After shaving fur, the rats were anaesthetized under light ether and 10mg of sterile cotton pellets were

---

Corresponding author - E-mail. pharmacobala@yahoo.co.in

Table I

Anti-inflammatory Activity of Methanol Extract of *Pisonia grandis* Leaf.

Treatment	Dose	Carrageenin induced paw oedema		Cotton pellet induced granuloma	
		Paw Volume (ml)	% Inhibition	Weight of the Cotton pellet	% Inhibition
Control	3 ml / kg	0.684±0.006*	-----	39±0.42*	-------
Extract	200m / kg	0.575±0.015*	15.90	35.41±0.16*	10.60
Extract	300mg/kg	0.425±0.009*	37.80	29.87±0.34*	24.19
Extract	500mg/kg	0.352±0.019*	48.53	24.32±0.23*	38.61
Phenylbutazone	100mg/kg	0290±0.008*	57.60	19.19±0.43*	51.50

* Significant at $P < 0.01$, P-value was calculated by comparing with control by ANOVA followed by Dunnett's test, Values are expressed as mean ± SEM.

inserted, one in each axilla of the rats. Extract (200,300 & 500 mg / kg), phenylbutazone (100 mg / kg) and control vehicle were administered orally for seven consecutive days from the day of cotton pellet implantation .The animals were anaesthetized on eighth day and cotton was removed surgically and made free from extraneous tissues. The pellets were dried to constant weight at 60°c[7].

Statistical Analysis

The results were expressed as mean± S.E.M. The significance statistical analysis was performed by ANOVA followed by Dunnett's test and p values $P<0.01$, implied significance[8].

RESULTS AND DISCUSSION

The present investigation was carried to evaluate the anti-inflammatory effect of *Pisonia grandis* in animal models and results are presented in Table-1.The preliminary phytochemical screening of methanol extract showed positive results for alkaloids, flavonoids, tannin and resin. The results revealed that the methanol extract 300 mg / kg inhibited the paw oedema volume at maximum 48.5 % at 4hr. after administration, while standard drug phenylbutazone exhibited 57.6 %.The methanol extract tested doses of 200 mg / kg and 300 mg / kg produced significant reduction in granuloma weight ,when compared to control group. These experiment results have established a pharmacological evidence for the folklore claim of the drug to be used as an anti-inflammatory agent. The exact mechanism of anti-inflammatory activity of extract is yet to be elucidated.

REFERENCES

1. The Wealth of India. CSIR, New Delhi; Vol. VIII, 1965. p.119-120.
2. Kirtikar KR. and Basu, BD. Indian Medicinal Plants, New Delhi: Jayyed Press; 1987. p. 119-120.
3. Nadkarni KM. India material Medica, 3rd ed, New Delhi: Popular Prakashan; 1954. p. 972.
4. Turner RA. Screening Methods of Pharmacology, Academic Press, Newyork; 1965 .p.22-41.
5. Kokate CK. Practical Pharmacognosy, 4th ed, NewDelhi: VallabhPrakashan; 1994. p.107-111.
6. Winter CA. Risely EA and Nuss GW. Carregeenin induced oedema in hind paw of the rat as assay for Anti-inflammatory drugs. Exp Biol Med. 1962; 111: 544.
7. Winter CA. and Porter CC. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities in hydrocortisone ester. J Amer Pharmacol. 1957; 46:515.
8. Woodson RF. Statistical Method for Analysis of Biochemical data, Newyork: Wiely; 1987. p.315.

The Pharmacist 2 (2) 13-14 (2007)

Effect of Rosiglitazone on Fluid and Electrolytes Transport in Jejunum and Colon of Rat *in vivo:* - A Possible Mechanism of Action

Sanjiv Singh*, Jitendra Malik and B.M. Patil
KLE's College of Pharmacy, Belgaum, Karnataka 560041

ABSTRACT:

Rosiglitazone in intestinal mucosa under chronic inflammatory condition and acute inflammatory condition increases prostaglandin production. The result of our study suggests that Rosiglitazone (PPAR-y agonist) inhibited the fluid and electrolyte (Na and Cl^{-}) absorption in rat jejunum and colon. Further the ability of Indomethacin to abolish this effect of Rosiglitazone suggests that prostaglandins are involved in Rosiglitazone inhibiting fluid and electrolyte transport in jejunum and colon.

Keywords: *Rosiglitazone, electrolyte transport*

INTRODUCTION

Rosiglitazone, Thiazolinedione, Peroxisome proliferators- activated receptor-gamma (PPAR-y) activator, reduces insulin resistance in patients with type-2 diabetes[1]. Recent studies have shown the implication of the PPAR-y in control of inflammation and immune responses in colonic mucosa under the infl'J1llmatory condition by reducing COX-2 over expression and increasing prostaglandin E_2 production[2], while the role of prostaglandins in the regulation of intestinal fluid and electrolyte transport is well known[3]. Prostaglandins are normally synthesized by intestinal epithelial cells and appear to play an important role in physiologic regulation of intestinal fluid transport[4]. They do so by stimulating intestinal mucosal adenyl ate cyclase and thereby increasing cAMP concentration[5]. PGs when administered exogenously also cause intestinal fluid and electrolyte secretion in human as well as in experimental rat models[6]. Rosiglitazone also cause fluid retention and diarrhea in susceptible individuals[7]. However, there are no studies are reported the role of PPAR-^{3} agonist Rosiglitazone on fluid and electrolyte transport in intestine. In the present study we have evaluated the effects of Rosiglitazone on fluid and electrolyte transport in jejunum and colon in rat and determined the possible mechanism involved.

MATERIALS AND METHODS

Animals used

Wister rats of either sex weighing (150-18g) were used for study. All animal were maintained in the animal house at ambient temperature with food and water available *adlibitum*. Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Procedure

The animals were divided into four groups of 6 animals.

Group-1	Received Saline (control).
Group-2	Received Rosiglitazone (5mg/kg subcutaneous).
Group-3	Recieved Indomethacin (10mg/kg subcutaneous).
Group-4	Received combined treatment consisting Indomethacin (10mg/kg) and Rosiglitazone (5mg/kg)

Animal were deprived of food for 24 hrs before the experiement but with free access of water. The jlejunum and colon transport were determined simultaneously in the same animals by using modification of Beubler and Mascolo methods[8]. After anesthesia the abdomen was opened and a polythene catheter (no.8) was placed in the jejunum and fixed by ligation. A second ligature was tied approximately 20 cm distal end of the colon. The jejunum and colon were rinsed carefully with 10 ml to 20 ml of warm sterile 0.9% Nacl solution respectively to remove the contents followed by blowing air with syringe. After one hour of animal preparation prewarmed tyrode solution was administered in both jejunum and colon and catheters were withdrawn before tying off the proximal end. Indomethacin was administered 15 min after animal preparation.

**Corresponding author - E-mail: sajivpy2006@yahoo.com*

Table I
Fluid and Electrolyte transport in Rosiglitazone treated rats

Treatment	JEJUNUM			COLON		
	Vol. of fluid transported (µl)	Electrolytes conc.(m Eq /L)		Vol. of fluid transported (µl)	Electrolytes conc.(m Eq /L)	
		Na^+	Cl^-		Na^+	Cl
Control	- 640	118	105	- 918	109.5	94
Rosiglitazone(5mg/kg)	- 157	152	127	- 27	140	130
Indomethasine(10mg/kg)	- 603	101	94.83	- 1045	102.8	94.6
Indomethasin + RSZ	- 506	109	95	- 781	115	94.3

RESULTS AND DISCUSSION

Rosiglitazone (5mg/kg s.c.) significantly inhibited fluid and electrolyte (Na+ and Cl -) transport in both jejunum and colon. Under basal condition there was a net absorption of fluid, Na + and Cl - in both jejunum and colon. But after administration of rosiglitazone (5mg/kg subcutaneous) net fluid and electrolyte (Na+ and Cl -) absorption were reduced. Administration of Indomethacin (10mg/kg subcutaneous), 15 min prior to the administration of saline (subcutaneous) had no significant effect on jejunum and colon, fluid and electrolyte transport (Table No.1). Compared to control group fluid and electrolyte (Na+ and Cl -) content was not significantly different in this group but pretreatment of animals with Indomethacin (10 mg/kg subcutaneous) completely abolished rosiglitazone (5 mg/kg subcutaneous) induced inhibition of fluid and electrolyte transport in both jejunum and colon. (Table No.1).

ACKNOWLEDGEMENTS

The authors are thankful to K.L.E. Society, Belgaum for providing necessary facilities to carry out this work.

REFERENCES

1. Kanoh Y, Bandyopadhyay G, Sajan MP, Standaert ML and Farse RV·Thiazolidinedinedione treatment enhances insulin effects on protein kinase C-zetas/lambda activation and glucose transport in adipocytes of nondiabetic and Goto-Kokizaki type II diabetic rats. J Boil Chern. 2000; 275: 16690-16696.
2. Hiroyasu Inoue, Tadashi Tanabe and Kazuhiko Umesono. Feedback Control of Cyclooxygenase-2 Expression through PPARy. Indian Journal of Biological Chemistry. 2000; 275: 28028-28032.
3. Sanchez-hidalgo M, Martin AR and Villegas I. Rosiglitazone, an agonist of peroxisome prolifretor-activated receptor gamma, reduces chronic colonic inflammation in rats. Biochem. Pharmacolo. 2005; 69(12): 1733-44.
4. Tsurumi K and Fujimura H. Effect of anti-inflammatory drugs on endotoxin induced diarrhoea in mice. Jap J Pharmacol. 1983; 33:165-73.
5. Field M, Musch MW and Stojy JS. Role of prostaglandins in the regulation of intestinal electrolyte transport. Prostaglandins. 1981; 21: 73-79.
6. Robert A, Nezamis JE and Lancaster C. Enteropooling assay: a test for diarrhea produced by prostaglandins. Prostaglandins 1976 may; 11(5): 809-28.
7. www.netdoctor.co.uk medicines/100004447.html Dated Jan 10th 2005
8. Beubler E and Horina G. $5HT_2$ and 5HT3 receptor subtypes mediated cholera toxin-induced rat intestinal fluid secretion in the rat. 1990; 99: 89-92.

The Pharmacist 2(2) 15-16 (2007)

Antimicrobial Evaluation of the Leaves of *Ervatamia coronaria*

Navin R. Raj [1]*, Chanchal N. Raj [2], Pushpendra Kannojia[1], N. Balakrishnan[1] and A. B. Panda [1]
[1]Dept. of P'ceutical Chemistry, TIT Pharmacy Bhopal (M. P.)
[2] Bhabha Pharmacy Research Institute Bhopal (M. P.)

ABSTRACT

Antimicrobial activity of Voacristine, an Indole alkaloid isolated from the leaves of Ervatamia coronaria was studied against various Gram positive and Gram negative bacteria. By agar well diffusion method, the compound showed moderate inhibitory effect against both species of bacteria. The Anti microbial activity of Voacristine was compared (125 mg/disc) with the standard Antibiotic Ampicillin (10mg disc).

Key Word : *Antimicrobial Evaluation, Ervatamia coronaria*

INTRODUCTION

Ervatamia coronaria (Wall.) Coock,, Syn ;*Tabernamontana coronaria* (Family ; Apocynaceae)[1] is a shrub or small tree attaining a height upto 2.4 - 4.5 m. and distributed in open forest of Konkan , North Kanara, Western Ghats in Malabar and Travancore upto an altitude of 3000 ft. In Indian system of medicine the plant is used as an astringent, aphrodisiac, tonic, purgative[2]. It is effective as an anti-inflammatory, analgesic and useful in liver and spleen disease[3].Literature are available with respect to its phytoconstituents; however the detail phytochemical and biological evaluation has not been dealt in depth.

MATERIALS AND METHOD

Plant material

The leaves of *Ervatamia coronaria* was collected from Mangalore and identified by Dr. S. Gopalkrishna Bhat, Dept.of Botany, Poornaprajna College Udupi. The specimen sample is kept in the department. The leaves were dried under sun light and reduced to coarse powder.

Extraction and Isolation

Sun dried powdered leaves of *Ervatamia coronaria* (1.5 kg) were soaked with 25% aqueous ammonium hydroxide and exhaustively extracted with chloroform by soxhlet extraction method. The solvent was removed and dried in vacuum to get chloroform extract (35 gm.) which was further partitioned with dilute HCl (0.1 N) The aqueous layer was alkalized with Conc. ammonium hydroxide, which was further partitioned to give ether extract. The ether fraction was then concentrated to give alkaloidal fraction (6.35 gm.).The alkaloidal fraction (6.35 gm.) was adsorbed on a column of silica gel eluting with chloroform: methanol (50:50) as a solvent. The eluents collected were monitored by TLC technique and similar eluents were mixed together and evaporated, which was further confirmed as an Indole alkaloid Voacristine by spectral analysis.

Antimicrobial Activity

Modified Kirby - Bauer method[4] was used to evaluate the anti microbial activity of the compound.The anti microbial activity was tested by disc diffusion method.The Bacterial culture were grown in Mueller Hinton agar by transferring 25ml. of the media into pre sterilized petridish and allowed to solidify at room temp. for 1hr. Suspension of organism (10^6cell/ml.) were spread into nutrient agar. Sterile filter paper disc (6 mm.) impregnated with 10 µl of test sample (Voacristine) was placed on agar surface at equidistant and petri dishes with bacterial cultures were incubated at 37° C for 24 hrs. The paper disc of standard antibiotic Ampicillin (10µg/disc) was also incorporated in the petri dishes.

The inhibition zone diameter (IZD) was measured by deducting the disc diameter.

The anti microbial activity of test compound is shown in Table-I

**Corresponding author - E-mail: pharmanavin@rediffmail.com*

Table I

S.No.	Compound	Diameter of zone inhibition(mm.)			
		B. sub	*S. aur*	*E. coli*	*P. aer*
1.	Voacristine	14	13	16	12
2.	Ampicillin	16	16	17	15

RESULT AND DISCUSSION

Table I shows the moderate Anti microbial activity of Voacristine isolated from the leaves of *Ervatamia coronaria* when compared to standard drug at 10mg level.

REFERENCES

1. Chopra, Nayar and Chopra. Glossary of Indian Medicinal Plants. New Delhi: PID; 1956. p. 110
2. Kirthikar KR. and Basu BD. Text Book of Indian Medicinal Plants. Periodical Experts Book Agency;1991.p.1576-1578.
3. The Wealth of India-Raw Materials. Vol. III. New Delhi: C S I R; 1970.p.192-193.
4. Bauer A.W., Kriby W.M.H., Sherris J.C., and Trunk M. Antibiotic succceptibility testing by a standardized single disc method. Amer.J.Clin.Pathol. 1966; 45: 493.

The Pharmacist 2(2) 17-19 (2007)

Comparative Study of Polyherbal Formulation Cebrotone and Neurocetam for Neurocognition Enhancement

V. Khare*, Rewa Verma, P. Kannojia, B.J. Mishra and Richa Mishra.
Department of Pharmacology, Technocrats Institute of Technology-Pharmacy,
Bhopal (M.P.) kannu_16mar@yahoo.com

ABSTRACT

The present study was aimed at investigating the effects of Cebrotone, an Ayurvedic polyherbal preparation on memeory in mice. Drug Cebrotone was administered orally at a dose of 13.5mg/kg/day and was compared with effects obtained from Neurocetam administered orally at a dose of 3.5mg/kg/day (prescribed dose) in mice. Elevated plus maze apparatus served as the exteroceptive behavioral models for testing memory. Interestingly, both the marketed preparations enhances the memory but the herbal preparations due to its less unwanted effects may be useful for the management of loss of memory in various diseases.

Key words: *Cebrotone, ayurveda, Neurocetam, memory*

INTRODUCTION

Memory is only one aspect of cognitive functioning. Cognitive functioning refers to the collection of brain functions that constitute our intellectual ability. Included in this are higher order sensory and motor processing, attention and concentration, language function, and executive functions.[1]

Nootropics, popularly referred to as "smart drugs", "smart nutrients", "cognitive enhancers" [2] and "brain enhancers", are substances which claim to boost human cognitive abilities (the functions and capacities of the brain).[3,4] The word nootropic derived from the Greek words *noos*, or "mind," and *tropein* meaning "to bend/turn". Typically, nootropics are alleged to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth.[5]

The nature provides a new opportunity to regain one's full mental capacity. A number of herbs traditionally employed in the Indian system of medicine "Ayurveda", [7] have yielded positive results.[4]

The current study was aimed to investigate the effect of Cebrotone®, an Indian Ayurvedic Poly – herbal formulation on memory in mice. It has been clinically used as a memory enhancer drug. Formulation consists of Shankhapushpi, Brahmi[8], Ashwagandha, Malkangoni, Jatamansi, Vacha. The results obtained were compared with Allopathic preparation. Neurocetam® consists of Neurocetam.

MATERIALS AND METHOD

Test substance and drugs: Commercially available Ayurvedic formulation Cebrotone® (Mfd. by-Vasundhara Pharmaceuticals Ankleshwar, India.) was obtained from local stockiest, Bhopal, India. Neurocetam® (Mfd.by-Bangalore Pharmaceuticals & Research Lab (P) Ltd. Bangalore, India.) was purchased from the local registered chemists shop. Both the preparations were liquid syrups.

Animals: The experiment was carried out using male, Swiss Albino mice procured from the disease-free small animal house of the institute. Young mice (7-10month old) weighing around 25-30g were used in the present study. The animal had free access to food and water, and they were housed in a natural (12hr each) light-dark cycle. Food given to mice consisted of wheat flour kneaded with water and mixed with small amount of refined vegetable oil. The animals were acclimatized for at least 5days to the laboratory conditions before behavioral experiments. Experimental protocol was approved by institutional animal ethics committee and the care of lab animals was taken as per the guidelines of committee for the purpose of control and supervision of experiments on animal. (CPCSEA).

Method: In the present study, the mice were divided into different groups. Each group comprised of a minimum six animals. Cebrotone (13.5mg/kg/day)and Neurocetam (3.5mg/kg/day) was administered orally in mice. Mice were divided into 3-groups of six animals each as follows: Group I served as control and received distilled water (10ml/kg, p.o.).Group II animals received Cebrotone (13.5mg/kg/day) orally. Group III received Neurocetam (3.5mg/kg/day) for comparison. The Elevated plus maze[6] (EPM) apparatus used in the study consisted of two open and two closed arms facing each other. The maize was elevated at a height of 50cm from the ground .The animal were placed individually 30 min after oral administration of either vehicle or test drug at the end of either of the open arm and the time taken by the animal to move from open to closed arms was noted on the first day. The time elapsed between the time that the animal was placed on the open arm and the time at which all four legs were inside the enclosed arm, was noted as transfer latency.[9] The transfer latency was again recorded 24 hour after the first exposure.[10]

**Corresponding author - E-mail. kannu_16mar@yahoo.com*

RESULTS AND DISCUSSIONS

The mice showed a significant decrease in transfer latency in all groups (including control) on the third day. The analysis revealed significant differences in transfer latency in EPM performance between Cebrotone and distilled water treated animals on all the three days. The results were comparable with the allopathic formulation of Neurocetam.(Figure 1).

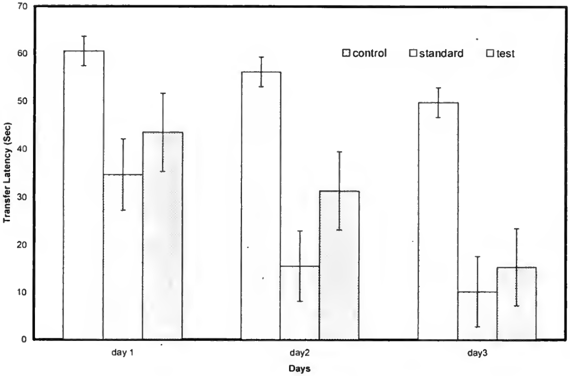


Figure 1: Effect of Cebrotone® (CT) and Neurocetam® (NC) on spatial memory in EPM in mice

Nootropics represents a new class of psychotropic agents with selective facilitatory effect on integrated functions of the central nervous system, particularly on intellectual performance, learning capacity and memory . Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis affect day-to-day life.[11] A number of drugs have now been introduced in therapy to ameliorate cognitive deficits.

Neurocetam, the first representation of a class of nootropic agents, has been shown to improve the condition with memory deficit generally seen in geriatric individuals and further Neurocetam administration had improved learning abilities and memory capacities in laboratory animals .In patients with Alzheimer's disease,[12] immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain. Since inflammation can cause damage to brain tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease and is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in patients with Alzheimer's disease. Moreover, it has also been observed that upon chronic use of anti-inflammatory drugs in elderly patients suffering from Alzheimer's disease showed reduction in symptoms.

Brahmi is an ayurvedic drug and is also have the ability to increase the memory capacities in laboratory animals. Along with brahmi, shankpushpi and ashwagandha are also seen to increase the memory capacities. As Neurocetam has more better effect than other polyherbal drugs, but is also associated with certain adverse effects like: - insomnia or somnolence, weight gain hyperkiesia, nervousness, depression etc.

REFERENCES

1. Wood AJJ. Drug Therapy: Alzheimer's Disease. N. Engl. J. Med. 2004; 351: 56-67.
2. Prakash JC, Sirsi M. Comparative study of effects of Brahmi and Chlorpromazine on motor learning in rats. J. Sci. Indust. Res. 1962; 21: 93-96.
3. Singh HK, Dhawan BN. Effect of *Bacopa monniera* Linn. (Brahmi) on avoidance responses in rat. J. Enthnopharmacol. 1982; 5: 205-214.
4. British Herbal Pharmacopoeia. London: British Herbal Medicine Association, 1996.
5. Parle M, Dhingra D, Kulkarni SK. Neuromodulators of learning and memory. Asia Pac. J. Pharmacol. 2004; 16: 89-99.
6. Kulkarni SK. In: Handbook of experimental Pharmacology. 3rd ed, Delhi: Vallabh Prakashan; 1999.
7. Tripathi B. Charak Samhita. Vol. 2. 3rd ed. Varanasi: Chaukhamba Surbharati Prakashan; 1994.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. 3. Dehradun: International Book Distributors; 1998.
9. Parle M, Dhingra, D, Kulkarni SK. Improvement of mouse memory by *Myristica fragrans* seeds. J. Med. Food. 2004; 7: 157-161.
10. SinghHK,DhawanBN. Neuropshycopharmacological effects of the Ayurvedic Nootropic *Bacopa monneiri* Linn. (Brahmi). Indian J. Pharmacol. 1997; 29: 359-365.
11. Wenk GL. Current protocols in neuroscience. New York: John Wiley & Son's; 1998.
12. Vasudevan M, Parle M. Pharmacological actions of *Thespesia populnea* relevant to Alzheimer's disease. Phytomedicine. 13: 677-687.

The Pharmacist 2(2)21-23 (2007)

Formulation and Evaluation of Transdermal Patch of Colchicine with Release Modifiers

Kuldeep Ganju*, Avinash Kondalkar*,and A.K.Pathak
Department of Pharmacy, Barkatullah University Bhopal 462 026

ABSTRACT

Matrix type transdermal patches of Colchicine were fabricated using the polymer, polyvinyl alcohol in 5% and 10% concentrations. The change in polymer concentration also changes their physical properties like tensile strength, thickness, moisture absorption etc. In the present study the in vitro drug release was studied using various release modifiers like myristic acid and succinic acid. The transdermal patch with myristic acid as release modifier, showed the increased permeation of the drug, whereas the transdermal patch with the succinic acid showed decrease in drug release through the matrix.

Key Words: *Transdermal patch, Polyvinyl Alcohol, Release modifiers, Succinic Acid and Myristic Acid*

INTRODUCTION

Colchicine is obtained from the fully developed dried whole corns, collected before flowering. The clochicine has a specific clinical effect in the treatment of acute gout. Colchicine can be administered both orally and intra-veinously. Its anticancer properties are attributed to its ability to bind itself to tubulin, the protein subunit of microtubules. A combination of vit-E with colchicine may also be effective in ameliorating injury of lungs caused by herbicides. Colchicine significantly lowers total lipids and cholesterol. It also has anti mitotic activity[1]. Colchicine occurs in the form of pale yellow crystals, amorphous scales or powder. It is freely soluble in water and ethnol(95%) but slightly soluble in ether[2]. Colchicine melts at about 145°C [3]. To bypass the first pass hepatic metabolism which degrades the potency of the molecule, transdermal patch of Colchicine incorporated with release modifiers were prepared to facilitate the drug release, has been formulated.

MATERIALS AND METHODS

Reagents

Colchicine (CDH lab.), ployvinyl alcohol(PVA), Glycerine, Phosphate Buffer 7.4 pH , myristic acid, succinic acid.

Preparation of Matrix Diffusion Patches of Colchicine

The solvent-casting technique employing a mercury substrate was used to formulate the poly vinyl alcohol patch, containing colchicines. Patches with varying concentrations of polymers were prepared along with release modifiers such as succinic acid and myristic acid. The polymeric solution was prepared by dissolving the PVA in HPLC grade water using glycerin as plasticizer. A measured volume of each of the polymeric solutions was poured into mould and dried at room temperature. To prevent the fast evaporation from the patches a funnel was placed inverted on the mould. After ensuring the complete evaporation of the solvent, patches of 3.0 cm diameter were cut and packed in aluminum foil and stored in desiccators for future study.

Physical Characterization

Thickness of polymeric patch was measured by using a screw gauge , having least count of 0.001 mm. In order to determine the elongation in terms of tensile strength, the polymeric patch was pulled by means of a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation *i.e.* the distance traveled by the pointer before break of the patch was noted . The tensile strength was calculated as kg/ cm^2. The presence of moisture may not affect the hardness of patch in the normal environmental conditions, but it may affect in exaggerated conditions. The polymer patches were cut, weighed and placed in a humidity chamber maintained at 68% RH for 72 hours to attain equilibrium. After 72 hours polymer patch were taken out and weighed accurately. The differences between initial and final weights were computed as percentage moisture absorbed. A weighed quantity of anhydrous calcium chloride was taken up to 10 mm height in each cell and a thin layer of silicon adhesive grease applied over the brim then patch was placed on brim and adhesive was allowed to set for 5 minutes. The cells were accurately weighed and kept in humidity chamber maintained at 68% RH for 24 h. After 24 h, the cells were again weighed and an increase in weight was considered as a quantitative measure of the moisture transmitted through the patch.

Skin Preparation:

The full thickness goat skin was washed with purified

**Corresponding author - E-mail: akondalkar@rediffmail.com* water after removal of all subcutaneous fat and hairs and cut in to pieces for experimental use. The skin pieces were soaked in Phosphate buffer and stored at temperature 1-3°C until used.

***In vitro* Drug Permeation Studies:**

A comparative drug permeation study was carried out with drug solution in Phosphate buffer pH 7.4 and stable patches through goat skin using modified diffusion cell[4]. The concentration of drug was kept similar in drug solution in Phosphate buffer and the patches to compare permeation profile. The patches and drug solution were kept on stratum corneum side of cells and this patch - skin - complex sandwiched between donor and receptor compartment. The receiving compartment contains blank 10.0 ml of Phosphate buffer pH 7.4 and touches the dermal side of the skin. The whole of the assembly was kept on magnetic stirrer, which was thermostatically controlled at 37± 2°C at 100 rpm. Samples were withdrawn at pre set time interval from the receiving compartment and analyzed spectrophotometrically at 294 nm using JascoV-530 Germany double beam spectrophotometer The permeation studies were determined for period of 9 hrs. and calculated as cumulative percent drug released.[5]

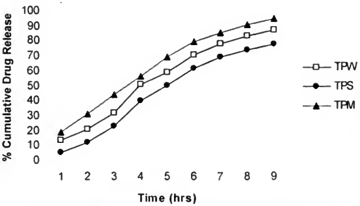


Figure 1. In-Vitro permeation profile of colchicine from TPW, TPM & TPS Patches

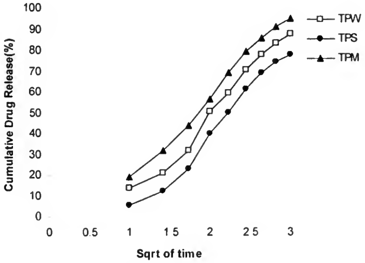


Figure 2. In-Vitro permeation profile (Higuchi Model) of colchicine from TPW, TPM & TPS Patches

Table 1
Physical Parameters of Transdermal Patches of Colchicine

Formulation Type	Polymer used	Modifier Used	Thickness (mm)	Tensile Strength(kg/cm^2)	Moisture absorption(%)
TPW (5%)	PVA	Without modifier	0.042±0.0012	1.39±0.013	2.87±0.14
TPW(10%)	PVA	Without modifier	0.044±0.0023	1.42±0.024	3.33±0.52
TPS(5%)	PVA	Succinic acid	0.039±0.0017	1.37±0.031	3.21±0.31
TPS (10%)	PVA	Succinic acid	0.038±0.0016	1.45±0.021	3.58±0.31
TPM (5%)	PVA	Myristic acid	0.041±0.0015	1.40±0.12	2.92±0.61
TPM (10%)	PVA	Myristic acid	0.043±0.0034	1.49±0.051	3.86±.35

RESULTS AND DISCUSSION

The primary objective of this study is to develop a novel transdermal delivery system (patches) of colchicine, which can eliminate the problems associated with the conventional routes of administration of the drug. In the present work transdermal patches of colchicine with various polymeric concentrations and various release modifiers were prepared. The effect of varying concentrations of the polymer on the physical properties of the transdermal patches was studied. The physicochemical characteristics with respect to thickness, moisture absorption and tensile strength of the transdermal patches of were satisfactory Table 1 The higher polymeric concentrations showed good film forming properties.

To study the effect of release modifiers on rate of drug diffusion, various release modifiers were incorporated to the transdermal patch and subjected to *in vitro* diffusion studies.

The *in-vitro* release data from the most stable patches Transdermal Patch without modifiers(TPW), Transdermal Patch with Succinic Acid as modifier (TPS) and Transdermal Patch with Myristic Acid as modifier (TPM) in terms of thickness, tensile strength and water transmission, followed zero order. The kinetic treatment revealed that the drug release from the patches followed Higuchi Model, as the correlation coefficient of linear relationship between the cumulative percent drug released and the square root of time of TPM was found to be 0.9956 and for TPW it was 0.9931, where as the correlation coefficient of linear relation for zero order was found to be 0.9812 for TPS. Thus the comparison of coefficient of linear relationship between the zero order and Higuchi model suggests that the drug permeation is controlled by diffusion in the transdermal patches (Figure 1 & Figure 2).

REFERENCES

1. The wealth of India. Vol II .New Delhi: CSIR; P-150-53.
2. Indian Pharmacopoeia. Vol I. N Delhi: Ministry of Health and Family Welfare; 1996.p. 204.
3. Remington.The Science And Practice Of Pharmacy. 20 edn. p. 1462.
4. Wester RC. Maibach H.I: Percutaneous absorption in the rhesus monkey compared to man. Toxicol. Appl.Pharmacol. 1975; 32: 394 .
5 Deepak Gondaliya and Kilambi Pundarikakshudu. Studies in Formulation and Pharmacotechnical Evaluation of Controlled Release Transdermal Delivery System of Bupropion. AAPS Pharm Sci Tech. 2003; 4(1): 3.

The Pharmacist 2(2)25-27 (2007)

Preliminary Pharmacognostic Evalutions and Phytochemical Studies on Leaf of *Chenopodium album* (Bathua Sag)

M.S.Pande*and A.K. Pathak
Department of Pharmacy, Barakatullah University, Hoshangabad road,
Bhopal (MP) 462026

ABSTRACT

The leaves of Chenopodium album Linn (Chinopodiaceae) are reported to have great medicinal value. Pharmacognostic evaluation including examinations of morphological and microscopic characters, determination of leaf constant, ash value, powder analysis, and extractive values were carried out. Phytochemical screening including qualitative chemical examinations was also carried out.

Key Words : *Chenopodium album, Bathua, Phytochemical, soxhlet extraction, phytoconstituents, pharmacognostic.*

INTRODUCTION

A genus of strong smelling herb commonly known as pigweed in English and distributed through out world. About 21 species occur in India of which few have been introduced. Some are cultivated for vegetable, and few for grains. In Hindi common name is Bathua sag. A polymorphous, mealy white, erect herb, up to 3.5m in height, and found wield in altitude of 4,700 meters and cultivated through out India. Stems rarely slender, angled, often striped green, red or purple; leaves rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 10-15 cm long, petioles often as long as thick blade; flowers in clusters forming a compact or loosely panicle spikes in axils; utricles with round, compressed, shining black seeds, possessing sharp margins.

The herb is a common weed during summer and winter in waste places and in the field of wheat, barley, mustard, gram and reduces their yield. The weeds are low growing while the cultivated plants are tall and leafy. The young plant of not more than 20 cm is much esteemed as a potherb. The tender shoots are eaten raw in salad or with curd; they are also cooked as vegetable or the cooked shoots are mixed with curd and eaten. The dried herb is stored for future use. It is also used as fodder; pigeons consume the plant in large quantities. The leaves are rich in potassium and vitamin C.

The leaves of *Chinopodium album* known as bathua sag is being used in traditional medicines. It has been found to have antipruritic and antinociceptic [2], sperm immobilizing agent [3], cryptomeridiol and 8- alpha-acetoxycyryptomeridiol as growth promoting activity. It has been found to have flavonoid as phenolic amide[5], hypotensive in activity[6], saponin[7], rich in iron content[8], cinnamic acid amide[9], alkaloid chinoalbicin[10], apocortinoid[11], xyloside[12], phenols and lignans[13].

MATERIALS AND METHODS

The leaves of *Chenopodium album* Linn (Chinopodiaceae) collected in and around Bhopal were identified in Department of Pharmacy, Barakatullah University, Bhopal. A voucher specimen (No BUPH/4041C) was deposited in the department. The plant was morphologically examined for shape of leaves, apex, base, margin etc. A separate section was prepared and examined for the identification of starch grains by staining with iodine solution. Powder (# 60) of the dried leaf was used for the observation of powder microscopic characters. The powder drug was separately treated with phloroglucinol –HCL solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains [14] as a part of quantitative microscopy, stomatal number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of plant [15].

Total ash, water and alcohol soluble ash, sulphated ash was also determined. Alcohol and water-soluble extractive values were determined [16].

Preliminary Phytochemical studies

The powder of dried leaves was subjected to continuous soxhlet extraction with various organic solvents such as petroleum ether (60-80° c), chloroform, benzene, methanol & ethanol respectively. After concentration and drying of each extract in vacuum desicator identification of phytoconstituents was carried out using thin layer chromatography method by different detecting reagents [17].

**Corresponding author - E-mail: milindpandey2006@rediffmail.com*

Table 1
Successive solvent Extraction of leaves of *Chenopodium album* Linn

S. No.	Solvents used	Colour & Consistency	Average extractive values in % w/w on dry weight basis
1	Petroleum Ether 40-60	Black green oily mass	3.53
2	Benzene	Black Green sticky mass	2.33
3	Chloroform	Light green residue	2.83
4	Acetone	Yellow	2.66
5	Methanol	Yellow blackish mass	5.44
6	Ethanol	Brown dry mass	4.55

Table 2
Thin layer chromatography scheme used to detect various extracts of leaves of *Chenopodium album* Linn

Solvent system used	Detection Reagent	Observation	Inference	P	B	C	A	M	E
Ethyl acetate: Methanol: Water (75.5:13.5:10)	KOH	Red. (Vis)Yellow	Anthraquinone Anthrone	-	-	-	-	-	-
	Vanillin sulphuric acid	Red/ yellow/brown /blue-green	Bitter principle	-	-	-	-	-	-
	Dragendorffs reagent	Orange Red (vis)	Alkaloid	-	-	+	+	+	-
	NP/PEG and UV	Yellow/green /orange	Flavonoid	-	-	+	+	-	+
	VS reagent	Blue (vis)	Saponin	-	-	-	-	-	-
Toluene : ethyl acetate (93: 7)	VS reagent	Red/ yellow/ brown/blue-green	Essential oil	+	+	-	-	-	-
	Hcl/Acetic acid	Blue brown	Valepotriate	-	-	-	-	-	-
	NH3 / KOH	Light Blue brown	Coumarin	-	-	-	-	-	-

Table 3
Evaluation of *Chenopodium album* Linn leaves

S. No.	Parameters	Values obtained w/w on dry weight basis
1	Ash value	9.55
2	Water soluble ash	3.85
3	Acid Insoluble ash	8.33
4	Alcohol soluble ash	7.28
5	Sulphated ash	10.11
6	Stomatal number	20-23
7	Stomatal index	4.9-8.8
8	Veinislet number	8-11
9	Veinlet Termination No	5.5-7
11	Palisade ratio	9.5-11.9

RESULTS AND DISCUSSION

The morphological studies revealed the shape of leaves of *Chenopodium album* Linn simple, rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 11-14 cm long, petioles often as long as thick blade, lanceolate to oblong and length varies from 9 cm to 4.5 cm broad with dentate margin. It has been found in dark green colour with smooth under surface. The petioles were of 1 to 1.3 cm in length.

In powder microscopy straight walled polygonal collenchyma and yellow coloured bean shaped mass with mesh like striations were observed prominently. Multicellular covering type of trichome distinctly observed with bunch of parenchymatous polygonal cells were observed. A single piece of xylem and phloem with centrally located cambium layers were seen clearly.

In the TS of leaves of *Chenopodium album* Linn showed the presences of circular arch of xylem covered with phloem. Thick walled parenchymatous cells were observed in upper epidermis but in lower epidermis replaced by round thin walled collenchyma. Further the multicultural covering trichome was also observed on horizontal layers of parenchyma.

Successive solvent extraction values in various organic solvent were observed as petroleum ether 3.53%, benzene 2.33%, chloroform 2.83%, acetone 2.66%, methanol 5.44% and ethanol 4.55% as shown in (Table No. 1)

The preliminary phytochemical studies with help of Thin Layer Chromatography method revealed that presence of alkaloid in chloroform, acetone, and methanol extract prominently. The flavonoid was present in chloroform, acetone, and ethanol respectively. The essential oil was observed in petroleum ether and benzene extract clearly.

The proximate analysis revealed that total ash 9.55, water soluble ash 3.85, acid insoluble ash 8.33, alcohol soluble ash 7.28, sulphated ash 10.11, stomatal no 20-23, stomatal index 4.9-8.8, veinislet no 8-11, veinislet termination no 5.5-7 and palisade ratio 9.5-11.9 values were observed in fresh leaves.(Table III)

REFERENCES

1. Kirtikar, K. R. and Basu, B. D. Indian Medicinal Plants: Lalit Mohan Basu, Allahabad, Vol III, 2nd ed. 1935. 1964-1965.
2. Dai, Y.; Ye, W. C.; Wang, Z. T.; Matsuda, H.; Kubo, M.; But, P.P.H Antipruritic and antinociceptive effects of *Chenopodium album* L. in mice. Journal of Ethnopharmacology. 2002; 81(2): 245-250.
3. Kumar S, Biswas S, Mandal D, Roy H. N., Chakraborty S, Kabir S.N., Banerjee S, Mondal N.B. *Chenopodium album* seed extract: a potent sperm-immobilizing agent both in vitro and in vivo: Contraception. 2007; 75(1): 71-8.
4. Bera, B.; Mukherjee, K.K.; Ganguly, S.N. Chemical investigation of the seeds of diploid cytotypes of *Chenopodium album*: Fitoterapia. 1991; 62(2): 178.
5. Horio, T.; Yoshida, K.; Kikuchi, H.; Kawabata, J.; Mizutani, J. A phenolic amide from roots of *Chenopodium* album: Phytochemistry. 1993; Vol. 33(4): 807-808.
6. Gohar, A. A. and Elmazar, M.M.A. Isolation of hypotensive flavonoid from *Chenopodium* species growing in Egypt: Phytotherapy Research. 1997; 11 (8): 564-567.
7. Lavaud, C.; Voutquenne, L.; Bal, P.; Pouny, I. Saponins from *Chenopodium album:* Fitoterapia. 2000; 71 (3): 338-340.
8. Yadav, S. K.; Sehgal, S. In vitro and in vivo availability of iron from Bathua (*Chenopodium album*) and spinach (*Spinacia oleracea*) leaves: *Journal of Food Science and Technology*. 2002; 39(1): 42-46.
9. Cutillo, F.; Abrosca, B.; Dellagreca,M.; Marino, C.D.; Golino, A. ; Previtera, L.; Zarrellia, A. Cinnamic acid amides from *Chenopodium album*: effects on seeds germination and plant growth: 2003; Phytochemistry. 64 (8): 1381-1387.
10. Cutillo F, D'Abrosca B, DellaGreca M, Zarrelli A. Chenoalbicin, a novel cinnamic acid amide alkaloid from *Chenopodium album*: Chem Biodivers. 2004; 1 (10): 1579-83.
11. DellaGreca M, Di Marino C, Zarrelli A, D'Abrosca B. Isolation and phytotoxicity of apocarotenoids from *Chenopodium album*: J Nat Prod. 2004; 67(9): 1492-5.
12. Dellagreca M, Previtera L, Zarrelli A. A new xyloside from *Chenopodium album* : Nat Prod Res. 2005;19 (1): 87-90.
13. Cutillo F, DellaGreca M, Gionti M, Previtera L, Zarrelli A. Phenols and lignans from *Chenopodium album*: *Phytochem Anal*. 2006Vol. 17(5): 344-9.
14. Khim (1982) Prir. Society, 396, Chemical Abstract 1982; 97, 159495q.
15. Anonymous Pharmacopoeias of India: Controller of Publication of Govt. of India, New Delhi. Vol II, 2nd ed1985: A 88 –90.
16. Kokate C.K () Practical Pharmacognosy: Vallabh Prakashan, New Delhi, Vol. I, 3rd ed.. 1994: 115-117, 123,124,127.
17. Wagner, H. () Plant Drug Analysis, Berlin: Verlas Publication; ,Vol. 1, 1989. pp.194, 291-304.

The Pharmacist 2(2) 29-31 (2007)

After Use Wastage of Semisolid Tube Packaging: A Study

Archana Singh, Rajesh S. Jadon*, D.C. Goupale and S. Nayak
Bansal College of Pharmacy, Bhopal, M.P.- 462021

ABSTRACT

Packaging used to contain, protect, and preserve products during their distribution, storage and handling. In this study, percent wastage was calculated after the complete evacuation of the collapsible tube. It was found that smaller packing have more percentage of wastage as compare to the larger packing. It was also observed that aluminum tubes retain more content as compared to plastic tubes & results in more wastage. The tubes having narrow opening has more wastage compared to wider mouth.

***Key Words:** - Packaging, wastage, semisolids collapsible tubes*

INTRODUCTION

Packaging, the technologies used to contain, protect and preserve products throughout their distribution, storage and handling, and at the same time to identify them, provide instructions for their use, and promote them. The packaging of pharmaceuticals involves containment as well as protection from potentially damaging environmental factors such as moisture, oxygen, temperature, and light.[1] The estimated value of packaging materials and machinery used for packaging throughout the world is £270 billion, of which the European figure is an estimated £100 billion. Some 60 per cent of all packaging is used for food and drinks, and it is also essential for cosmetics, domestic chemicals and electrical goods, pharmaceuticals, health care products, agricultural chemicals, seeds, and feeds, and industrial goods of all types, such as motor parts, and computer hardware and software.

Pharmaceutical semisolid preparations include ointments, pastes, cream emulsions, gels, and rigid foams. Their common property is the ability to cling to the surface of application for reasonable duration before they are washed or worn off. This adhesion due to their rheologic behavior, which allows the semisolids to retain their shape and cling as a film until acted upon by outside force, in which case they deform and flow.[2]

Packaging of semisolids consists of the packaging of oral liquids (solutions, syrups, emulsions, and suspensions) and products used for topical applications, such as ointments, gels, emulsions, suspensions, creams, & suppositories. Most often the semisolids used for topical purpose are packaged in collapsible tubes, jars, glass, and plastic bottles, with optional dispensing closures, which may include flip-top caps, dropper assemblies, medium-viscosity pumps (as used for products such in hand and body lotions), and high-viscosity pumps (as used for tooth gels). Pouches of assorted materials and shapes are commonly used for single-dose and sample dispensing.[3]

Each packaging form must be carefully evaluated in light of dosage form to give guidance for the direction of the package qualification. For example, packaging of sterile liquids for injection requires a very thorough test protocol. The packaging of the most semisolids involves test protocols that are closure to those for solids.[4] The main technical issues involve adequate demonstration that interaction between contact surface and product are not significant and that product containment and protection is adequate. The challenge to package developer for these types of dosage forms is the need to device test that will be effective in discriminating among material choice on an accelerated basis so that real-time stability studies can be initiated at the earliest point in the project. When properly done, such protocols will yield reliable results that shorten developmental time, reduce project costs, and minimize material costs.[5]

The aim of this study was just to find out the percentage of drug which is wasted as wastage due to incomplete content removal from the container.

MATERIALS AND MEDHODS

In this study various semisolid formulations packed in collapsible metal or plastic tube were collected and weighed accurately before and after complete evacuation of the container. Percent wastage was calculated on the basis of the amount retained in the container after complete evacuation.

**Corresponding author - E-mail. rajeshjadon@gmail.com*

Table 1
Percent Wastage of 5-10g Packing Size

Brand Name	*Company*	*packaging material*	*Total Content*	*Contents Remain After Complete Evacuation(g)*	*Percent Wastage (%)*
Begay	Chirayu Pharmaceuticals	Metal	7g	0.8580 ± 0.007	12.25±0.065
Lexus petroleum jelly Lipguard	Zen Beauty Tech	Plastic	10g	1.8400 ± 0.007	18.40±0.071
Moov	Paras Pharmaceutical Ltd.	Plastic	5g	0.3520 ± 0.009	7.04±0.062
Neosporin.	Burrough Wellcome(India)Ltd	Metal	5g	0.5502 ± 0.008	11.04±0.032
Promise anticavity	Balsara Home Products Ltd.	Plastic	10g	1.5340 ± 0.007	15.34±0.063
Quadriderm	Zyg Pharma Ltd.	Metal	5g	0.4390 ± 0.005	8.78±0.043
Sensur	Gracewell Glenmark Pharmaceuticals Ltd	Metal	5g	0.5012 ± 0.007	10.02±0.045
Sensur	Gracewell Glenmark Pharmaceuticals Ltd	Metal	10g	0.6012 ± 0.008	5.12±0.081
Aderferin™	Gladerma India Pvt. Ltd.	Plastic	15g	1.7320±0.0050	11.54±0.07
Betadine	WinMedicare Pvt. Ltd.	Metal	15g	1.1050±0.0054	7.36±0.063
Candid-B	Glenmark Pharmaceuticals Ltd	Metal	15g	1.5870±0.0045	10.58±0.07
Efaderm	Gladerma India Pvt. Ltd.	Plastic	20g	2.4320±0.0074	12.16±0.069
Itch Guard	Paras Pharmaceutical Ltd.	Plastic	15g	1.2200±0.0076	08.06±0.067
Pepsodent Whitening	Hindustan Lever Ltd.	Plastic	20g	2.1020±0.0087	10.51±0.056
Ring Guard	Paras Pharmaceutical Ltd.	Plastic	12g	1.9840±0.0072	15.01±0.068
Tantum Gel	Elder Pharmaceutical Ltd	Metal	20g	0.9340±0.0082	14.67±0.061

Mean ± SD (N=3)

Table 2
Percent Wastage of 80-200g Size Packing

Brand Name	*Company*	*packaging material*	*Total Content*	*Contents Remain After Complete Evacuation(g)*	*Percent Wastage (%)*
Close-up Vitamin fluoride system	Hindustan Lever Ltd.	Plastic	80g	1.8420±0.003	2.30±0.02
Colgate strong teeth	Professional Oral Care Products Pvt. Ltd.	Plastic	100g	3.1290±0.005	3.12±0.02
Colgate Total	Colgate Pamolive (India) Ltd	Plastic	150g	3.8450±0.003	2.56±0.03
Gillette series Shave gel	Gillette India Ltd	Plastic	80g	1.8220±0.004	2.27±0.03
Pepsodent complete germi check	Hindustan Lever Ltd	Plastic	175g	4.5020±0.004	2.57±0.02
Pepsodent Regular	Hindustan Lever Ltd	Plastic	200g	4.5020±0.002	2.25±0.03

Mean ± SD (N=3)

RESULTS AND DISCUSSIONS

The most semisolid like ointments, creams, and gels are widely packed in a tube either of aluminium or plastic. Initially collapsible aluminium tubes were introduced and still it is used in variety of products. Nowadays these aluminium tubes are slowly replaced by plastic tubes due to their cost and elegancy. Both cosmetics and drugs are packed in a tube packing. These packaging system has lot of advantages in the direction of safety and stability of the product but a common problem is always observed while complete removal of contents at the end use of the product.

In the present study a number of tubes were c were of variety of product and sizes. It was observed that tubes with the packing size of 5-10 g (Table 1) has more unused wastage i.e. 5-18 % The tubes having size more then 80 g (Table 2) has considerably less wastage i.e. on an average 2 %. It was also observed that aluminium tubes retain more content as compared to plastic tubes & results in more wastage. The tubes having narrow opening has more wastage compared to wider mouth. From the above results it can be said that the customer or patient has to waste the product unwillingly & knowingly due to problems in complete removal of contents. Therefore there is need to modify the design of tube packing to improve the complete use of the product. A lot of work & research can be done by the pharmaceutical engineers / pharmacists to solve this common problem.

REFERENCES

1. Banker GS, Rhodes CT. Modern Pharmaceutics.3rd ed. NY: Marcel Dekker Inc.; 1996. p. 272, 682 & 713.
2. Allen LV, Popovich NG and Ansel HC. Ansel's Pharmaceutical Dosage formand drug delivery systems. 8th ed. London: Lippincott Williams & wilkins; 2006. p. 284-86.
3. Lachman L, Liberman HA and Kanig. JL The theory and practice of industrial pharmacy. 4 ed . Bombay: Varghese pub house; 1991. p. 711-732.
4. Cooper J. Plastic container for pharmaceuticals testing and control. Publ 4. Geneva: WHO offset; 1974. p. 156.
5. Leonard EA. Packaging: specifications, purchasing and quality control. 3rd ed. NY: Marcel Dekker; 1987. p. 289.

The Pharmacist 2(2) 33-35(2007)

Estimation of Levocetrizine UV/Vis Spectroscopic Method in Tablet Dosage From

Atul Tripathi, Prashant Ghode*, Hemant Dhongade and S.Nayak
Bansal College of Pharmacy Bhopal M.P. 462021

ABSTRACT

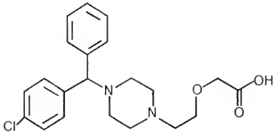
A simple, precise and sensitive UV Spectroscopic method in ultraviolet region has been developed for the determination of Levocetirizine in tablet dosage form. Levocetirizine exhibited maximum absorbance at 231 nm using methanol as a solvent. Beer's law obeyed in the range of 5-30 µg ml. Results of analysis of the methods were validated statistically and by recovery studies.

Key Words* : *Levocetrizine, UV Vis Spectroscopic studies

INTRODUCTION

Levocetirizine dihydrochloride is a third generation non-sedative antihistamine, developed from the second generation antihistamine cetirizine[1]. Chemically levocetirizine is the Acetic acid,[2-[4-[(*R*)-(4-chlorophenyl)phenylmethyl]-1piperazinyl]ethoxy]-, dihydrochloride[2] with molecular formula $C_{21}H_{25}ClN_2O_3.(HCl)_2$

The structural formula of levocetirizine is as follows[3]:



Levocetirizine dihydrochloride is used in several conditions such as allergic & perennial rhinitis, seasonal allergies, all forms of skin allergies like food & drug allergy or atropic dermatitis, chronic idiopathic urticaria.[1]

Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever[4, 5]. The extensive literature survey carried out revealed that no methods have been reported for the estimation of Levocetirizine by UV Spectrophotometer, whereas some methods for the estimation by capillary electrophoresis and some pharmacological studies were available[6, 7, 8].

MATERIALS AND METHODS

All the chemicals used were of analytical grade. Absorbance measurements and estimation were made with Ultra Violet Spectrophotometer -1700 by Shimadzu Co. Japan with 1.0 cm matched quartz cell and PMT as a detector. The gift sample of Levocetirizine was obtained from Zim Laboratories, Kalmeshwar, Nagpur (M.S.). The estimation of two marketed formulations was done by using the proposed method.

Selection of solvent:

Different solvents such as water, methanol and acetone have been tried and the individual spectrum was obtained. Maximum sensitivity was found with methanol. Hence, methanol was selected as solvent for present study.

Selection of Wavelength:

The spectrum of Levocetirizine (5ug/ml) was obtained. From the spectra the absorbance maxima for Levocetirizine was found 231nm. Hence, for present study 231nm was selected as λ_{max}

Preparation of sample Solution:

Twenty tablets each containing 5mg of Levocetirizine were weighed. The average weight was calculated. The tablets were grounded to fine powder and the quantity of powder equivalent to 5mg of Levocetirizine was weighed and dissolved in 20 ml of methanol in 50 ml of volumetric flask by gently warming and volume was made upto the mark. Further, 1ml was diluted upto the 10ml to get 10µg/ml.

Preparation of standard solutions:

100mg of Levocetirizine was taken in a 100ml volumetric flask. To this 20ml of methanol was added for dissolving the drug. It was shaken for one minute to get a clear solution and the volume was made up to 100ml

---

**Corresponding author - E-mail: ghoderprashant@gmailmail.com* with solvent (stock solution).10ml from stock solution was taken and again it was diluted upto 100ml.The final standard solutions were prepared in such a way that each standard flask contains 5,10,15,20 and 25 μg/ml of Levocetirizine.

Preparation of Calibration Curve: Aliquots of standard Levocetirizine were prepared in the concentration range of 5-25μg/ml and absorbances were measured at 231nm against methanol as a blank. The calibration curve was prepared by plotting absorbance Vs concentration. The concentration of sample was determined from calibration curve.

Estimation of Levocetirizine:

For Brand A (LCZ) the amount of Levocetirizine was found to be 5.056 mg/tab i.e. 101.12%.

For Brand B (Levcope) the amount of Levocetirizine was found to be 4.906 mg/tab i.e. 98.12%.

Table 1
Analysis of formulation

Formulation	Drug	Label claim (mg/tab)	Estimated amount (mg/tab)	%Label* Claim	% RSD*	% Recovery*
Brand A (LCZ)	Levocetirizine	5	5.056	101.12	0.751	100.45
Brand B (Levcope)	Levocetirizine	5	4.906	98.12	1.259	98.9

* **Mean of six determination**

Brand A: LCZ. Manufactured by Akumo drugs pharmaceuticals ltdMarketed by Repress Pharmaceuticals Pvt. Ltd., New Delhi

Brand B: Levcope. Manufactured by Next Wave India SirmourMarketed by Mankind Pharmaceutical Ltd., New Delhi.

Table-2
Optical characteristics, precision, accuracy data

Sr. no	Parameters	Values
1	λ_{max}	231nm
2	Beer.s law range(μg/ml)	5-25
3	Molar Absorbivity(l/mol/cm)	1.23×10^{4}
4	Correlation coefficient	0.9998
5	Slope	0.0867
6	Intercept	0.014

RESULTS AND DISCUSSION

Estimation of Levocetirizine in marketed dosage forms by UV Spectroscopic method which was carried out using optimized conditions. The assay procedures were repeated for five times and mean absorbivitis, mean absorbance, mean weight of standard drugs, weight of sample taken for assay was calculated. The percentage of drug, amount present in formulations and percentage RSD were calculated. Reproducibility of the method was validated with recovery studies and given in presented in Table 1. The results of analysis shows the amount of drugs was in good agreement with label claim of the formulations. Optical characteristics, precision and accuracy studies had been performed. Table 2. All the values were found to be well within the acceptable limits. The developed method is simple, accurate, sensitive, reproducible and rugged, hence can be used for the estimation of Levocetirizine in dosage forms and can also be used for routine laboratories purposes.

REFERENCES

1. Brunton LL, Goodman &Gilman's. The Pharmacological Basis of Therapeutics.11thed. New York: McGraw-Hill Medical Publishing Division; 2006. chap. 11.
2. Katzung BG. A Lange Medical Book Basic & Clinical Pharmacology. 10th ed. New York: McGraw-Hill Medical Publishing Division; 2007. 255.
3. The Merck Index.12th ed. Whitehouse Station (NJ): Merck and Co., Inc; 1996. 7000.
4. Tripathi KD. Essentials of Medical Pharmacology. 5th Ed. New Delhi (India).Japee brother's medical publishers Ltd; 2004. 135-155.
5. British Approved Names. British Pharmacopoeia Commission: 2002. 124
6. Van Eeckhaut A, Michotte Y. Chiral Separation of cetirizine by capillary electrophoresis. Medline. 2006 Jun; 27 (12): 2376-85.
7. Kapp A and Demarteau N. Cost effectiveness of levocetirizine in chronic idiopathic urticaria. Clinical drug investigation. 2006; 26(1): 1-11.
8. Hulhoven R, Rosillon D, Letiexhe M, Meeus MA, Daoust A, Stockis A. Levocetirizine does not prolong the QT/QTc interval in healthy subjects results from a thorough QT study. Eur J Clin Pharmacol. 2007; Sep 21:25-27.

The Pharmacist 2 (2)37-39 (2007)

Comparative *in vitro* Evaluation of Government Hospital Supplied and Commercial Brands of Paracetamol Tablets

A. Chourasia*, B. J. Mishra, Richa Mishra and P. Kannojia
Technocrats Institute of Technology-Pharmacy, Bhopal, Pin: 462021,
Madhya Pradesh, India

ABSTRACT

Two popular marketed brands of Paracetamol tablets A and B and Paracetamol obtained from two different Government hospitals C and D were evaluated comparatively for six in vitro parameters of tablet evaluation, viz. weight variation, friability, hardness, drug contents (assay), disintegration and dissolution. The results of study revealed that quality of hospital supplied Paracetamol tablets is comparable to that of the popular marketed brands of Paracetamol: irrespective of the general psychology that the quality of Government hospital supplied medicine is poor.

Key words: *Paracetamol, antipyretic, disintegration, dissolution, friability*

INTRODUCTION

Paracetamol is a non-steroidal analgesic and antipyretic agent[1] with minimal side effects. Chemically it is 4-hydroxy acetanilide (acetaminophen)[2]. Paracetamol is a potent antipyretic and has analgesic action almost equal to aspirin in therapeutic doses, commonly used in conditions like headache and muscular pain. It is the most frequently prescribed and one of the best drugs to be used as antipyretic. The clinical efficacy and safety of pharmaceutical products[3] are dependent upon the formulation parameters preset during its formulation to assure the desired purpose. The efficiency of pharmaceutical products depends on its formulation properties and manufacturing methods, hence it is likely that the quality of pharmaceutical product may vary. In India, large number of population receives the medication from Government hospitals[4], which is the most economical source of medicines. Therefore, the quality of medicines available in such hospitals is of utmost importance. It is a general psychology that the quality of medicines supplied by Government hospitals is poor as compared to marketed brands. A survey was conducted on 500 people in Bhopal city which led us to conclude that, 65% population prefers marketed brands of medicines over that of government hospital supplied medicines (Figure 1). Therefore, it was decided to carry out the comparative evaluation of Paracetamol tablets obtained from two different Government hospitals with that of most popular marketed brands. Paracetamol tablets with label claim 500 mg were taken for the study.

MATERIALS AND METHODS

Two commercially available brands of Paracetamol tablets were purchased from retail store and Paracetamol tablets of Government hospitals were collected. All brands had same manufacturing year, and were recently manufactured. The products were coded as A and B for commercial brands and C & D for hospital supply tablets. The products were evaluated for weight variation, hardness, friability, drug content (assay), disintegration and dissolution study in accordance with the Indian Pharmacopoeia (I.P. 1996). All the reagents and chemicals used were purchased from Qualigens (India) and were of AR grade. All the official evaluation tests[5] were carried out strictly in compliance with I.P.1996.

Dissolution test: Dissolution test was carried out using 900 ml of Phosphate buffer pH 7.8 as a dissolution medium, in a paddle type dissolution apparatus; make Labtech, at a paddle speed of 50 rpm for 30 min. Absorbances of the resulting diluted solutions were measured at 249 nm against phosphate buffer as a blank. The amount of dissolved drug was calculated using standard calibration curve. (Table 2)

Test for disintegration: One tablet was added in to each of the six tubes of the disintegration testing apparatus, make Labtech. The assembly was suspended in beaker containing 100 ml of water as the disintegration medium. The time required for complete disintegration of each tablet was observed and the average disintegration time was determined. (Table 1)

Assay: 20 tablets were weighed individually and powdered. Amount of powder equivalent to 0.15 gm of Paracetamol was taken with 50 ml of 0.1M NaOH and diluted with 100 ml of water in 250 ml volumetric flask and the volume was made with water, mixed and filtered. Accurately measured volume of 10 ml of this solution

---

**Corresponding author - E-mail: arvind_chourasia2000@yahoo.com*

was diluted to 100ml with water. To 10 ml of this solution 10 ml of 0.1 M NaOH was added and diluted to 50 ml with water and mixed. Absorbance of resulting solution was measured at 257 nm spectrophotometrically on a UV-Visible spectrophotometer, make Shimadzu, model no. Pharmaspec series UV-1700. (Table 3)

Hardness, Friability and weight variation[6]:

Hardness was determined using Pfizer hardness tester. Five tablets of each brand were tested for hardness. Friability of the tablets was tested using Roche type friabilator, make Labtech. For the friability testing, 20 intact tablets were weighed accurately and were placed in friabilator drum and rotated for 100 revolutions at speed of 2 revolutions per minute. The tablets were removed, de-dusted and weighed accurately. The percent friability was calculated using the following formula-

% Friability = I – F x 100/ I

Where, I = Initial weight, and F = Weight after friability.

Weight variation test was performed using 20 tablets on a top loaded digital balance, make Essae model no. DS-852J. Twenty tablets were weighed accurately and average weight was calculated. Each tablet was weighed individually and percent deviation from the average weight was calculated. (Table 3)

Table 1
Disintegration time for different Brands of Paracetamol Tablets

Average Disintegration Time (min: sec)	A	B	C	D
	8:32	4:15	6:32	4:08

Table 2
***In vitro* rate of dissolution of different brands of Paracetamol Tablets**

S. No.	Time of Analysis	Cumulative % Drug Release			
		A	B	C	D
1	10 min	73.00%	78.30%	71.68%	65.19%
2	20 min	78.30%	84.38%	80.20%	75.00%
3	30 min	92.00%	94.20%	95.7%	98.72%

Table 3
Results of weight variation, hardness, friability and assay of Paracetamol Tablets*

Brand	Hardness	% Friability	% Weight variation (± values)	Assay (%)
A	5.5	0.93 %	4.4%	101.46%
B	4.2	0.51 %	2.6%	89.67%
C	3.3	0.96 %	1.9%	100.525%
D	3.9	0.41 %	3.9%	91.53%

* Results are mean values of five replicate samples

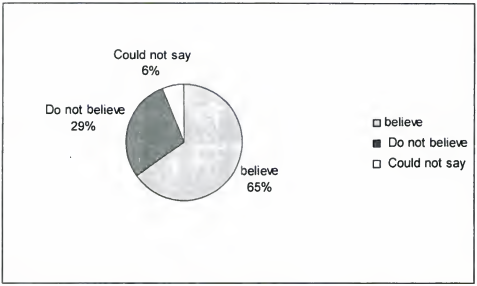


Figure 1: Survey Result of 500 persons in the region of Bhopal for faith in government hospital supplied medicine

RESULTS AND DISCUSSION

All the products complied with the standards for friability, hardness, weight variation, disintegration, dissolution and assay, except brand B which did not comply with the test for friability. The results are depicted in tables 1, 2, and 3. The dissolution pattern was found to be varying for each product, but within the prescribed limit. From the results obtained it is clear that, the overall quality of Government hospital supplied Paracetamol tablet is comparable with popular marketed brands.

This experimental study will help to change the view of people towards the Government supplied drugs. The study helped us to become aware that even the commercial brands of medicine may sometimes lack quality and fail some quality parameters set by the regulatory agencies.

The comparative study revealed that the hospital supplied samples did not comply with some of the parameters meant for the formulation of solid tablet dosage forms.

CONCLUSION

To conclude, the study helped us to know that the general mentality that the hospital supply of medicines throughout the country is poor compared to popular market brands needs to be changed. It is evident that the non government organizations and the government of state has taken serious steps to improve the quality of the medicines that are being supplied to the WHO aided hospitals. From the results of this study, the survey which was conducted before the start of the study could be proved wrong, as some quality parameters of popular market brands also did not match with the specifications meant for them.

REFERENCES

1. Burke A, Symth EM and Fitzgerald GA. Analgesic-Antipyretic agents; Pharmacotherapy of Gout. In: Brunton LL, Laso JS & Parker K editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill; 2006. p. 671-716.
2. Willette RE. Analgesic agents. In: Delgado JN & Remers WA editors. Wilson & Gisvold's Textbook of Organic Medicinal & Pharmaceutical Chemistry. 10th ed. Philadelphia: Lippincott & Raven Publishers; 1998. p. 687-726.
3. The Editors. The Practice of Medicine. In: Kasper DL, Hauser SL, Braunwald E, Longo DL, Jameson JL & Fauci AS editors. Harrison's Principles of Internal Medicine. 16th edition, Vol. 1, New York: McGraw-Hill; 2005. p. 1-6.
4. http://www.who.int/disease/
5. Govt. of India, Ministry of health and family welfare, The Indian Pharmacopoeia, Volume II, New Delhi: Controller of publication; 1996. p. A80 - A84.
6. Banker GS & Anderson NR. Tablets.In: Lachman L, Lieberman HA & Kanig JL editors. The Theory & Practice of Industrial Pharmacy. 3rd ed., Mumbai: Varghese Publication; 1991. p. 293-345.

The Pharmacist 2 (2) 41-44 (2007)

Topical Liposomal Gel with Aceclofenac: Characterization and *in vitro* Evaluation

Akanksha Garud* [1], Navneet Garud [2], Vivek Tomar [1] and Mayank Maheshwari [1]
[1]Institute of Professional Studies, College of Pharmacy, Gwalior-474001 (M.P) [2]School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior-474011 (M.P).

ABSTRACT

The objective of the present study was to design liposomal gel of Aceclofenac, a NSAID drug. Multilamellar liposomes were formed by lipid film hydration method using different ratios of phosphatidyl choline and cholesterol. The liposomal formulation was characterized for vesicle size analysis, entrapment efficiency, in-vitro permeation studies and in-vitro stability studies. The incorporation of bioadhesive gel Carbopol 974P further extended the release time of the liposomes. The comparative in-vitro permeation study of the liposomal suspension and liposomal gel showed that the drug permeation through dialysis membrane was greater than that of aqueous solution of drug and drug in carbopol gel. The stability studies depicted negligible drug loss at room temperature and in refrigerator.

Keywords : *Liposomes, Aceclofenac, Carbopol, Topical Application, stability*

INRTODUCTION

Liposomes are microscopic or submicroscopic structures, formed from phospholipids in the size range of 10 nm to 80 nm. Depending upon the method of preparation and size; liposomes may be referred as unilamellar and multilamellar phospholipids vesicles, comprising of one or more concentric lipid bilayers resulting in spherical particles[1,2] .Owing to the amphiphilic nature of the phospholipids, liposomes are suitable for sustained drug delivery of various lipophilic and hydrophilic drugs.

Since, the phospholipids correspond to the physiological membrane lipids, topical liposomes, in particular, possess greater penetrability through the skin[3]. This is the case for the inflamed skin, into which liposomes can penetrate readily. The use of liposomes to enhance penetration has been explored for oral, topical, ophthalmic and other parenteral routes since time back. Recently, liposomal carriers; have found application in topical formulations, avoiding the adverse effects associated with oral administration and also the parenteral administration which is restricted due to its aqueous solubility criterion[4].

The study aimed at preparing liposomal gel of Aceclofenac, for topical application[5]. The role of the bioadhesive polymer carbopol 974P was also observed in the in-vitro skin permeation studies. Aceclofenac, a NSAID drug is indicated for the relief of pain in osteoarthritis, ankylosis spondylitis, dental pain, low back pain, gynaecological pain, etc. Given orally, Aceclofenac shows various adverse effects viz. nausea, vomiting, and abdominal pain. The short half-life of 4-4.3 hr led to multiple dosing of the drug which further encouraged us to formulate liposomal gel of Aceclofenac for topical use as sustained drug delivery system[6].

MATERIALS AND METHODS

The drug Aceclofenac was obtained as a gift sample from Ind-Swift Pvt. Ltd.,Chandigarh. Carbopol 974P gift sample was procured by Colorcon Asia Pvt. Ltd.,Verna,Goa. Phosphatidyl choline (PC) and dialysis membrane bag (0.45 µm) was purchased from Hi-Media Laboratories, Mumbai. Propylene glycol was purchased from Qualigens Fine Chemicals,Bombay. Sodium lauryl sulphatewas purchased from CDH, New Delhi. All other chemicals and solvents used were of analytical reagent grade. Phosphate buffer pH 7.4 was composed of 137 mmolL^{-1} NaCl; 1.4 mmolL^{-1} KH_2PO_4 and 16.7 mmolL^{-1} Na_2HPO_4 in deionised water.

Preparation of Liposomes :

Multilamellar liposomes containing Aceclofenac drug were prepared by the lipid film hydration technique. The lipid phase was prepared by dissolving accurately weighed quantities of the drug, PC and Cholesterol in different ratios (Table 1) in methanol-chloroform mixture (1:2, v/v), in a round bottom flask. The organic solvent was removed from the lipid phase evaporation under vaccum at 45-55ÚC, to obtain a dry lipid film on the wall of the flask. The dry film was hydrated with phosphate buffer, pH 7.4 at temperature 60ÚC. The dispersion obtained was vortexed for about 2 min. After 24 hr, liposomal suspension was again washed with phosphate buffer pH 7.4 and centrifuged at 12000 rpm for 120 min, twice, in order to remove the unentrapped portion of the drug[7,8,9].

**Corresponding author - E-mail: akanksha.garud@gmail.com*

Preparation of gel :

For the incorporation of liposomes for topical delivery, a bioadhesive gel was made which acts as vehicle. Carbopol 974P (2 g) was dispersed in distilled water (88 ml) by stirring at 800 rpm for 60 min. Then, propylene glycol (10 ml) was added and the mixture was neutralized by addition of 10% NaOH. Mixing was continued until a transparent gel appeared. The amount of the base was adjusted to achieve a gel with pH 5.5.

Incorporation of liposomes into gel :

Liposomes containing the drug (separated from the entrapped drug) were mixed into the carbopol gel with an electrical mixer (25 rpm, 2 min).

Characterisation of liposomes :

Transmission electron microscopy (TEM): liposomes were analysed by placing the sample on copper grid and by negative staining with 2.0% phosphotungistic acid. Samples were dried at room temperature for 10 min and then analysed by TEM at 80 kV.

Vesicle size analysis: After hydration of liposomes with phosphate saline buffer at pH 7.4, followed by 2 min vortexing; the dispersion was observed under optical microscope at 65 X magnification. Liposomal size distribution of vesicles was noted using calibrated stage and ocular micrometer fitted in eye piece[10,11]

Vesicle count: Liposomal formulation was diluted upto 10 times phosphate saline buffer, pH 7.4 and the number of vesicle/mm^3 was counted by optical microscope using hemocytometer (CPF, China). The total number of vesicles/mm^3 was counted using the following formula:-

$$\text{Total no. of vesicles/mm}^3 = \frac{\text{Total no. of vesicles X dilution X 4000}}{\text{Total no. of squares counted}}$$

Drug entrapment efficiency: It is defined as the amount of drug associated with liposomes to the total amount of drug. To evaluate the loading capacity, the liposomal suspension was suitably diluted with phosphate saline buffer, pH 7.4 and centrifuged at 12000 rpm for 100 min. in Remi CPR-24 centrifuge. Pellets containing drug-loaded liposomes were formed at the bottom and the clear liposome-free solvent fraction (supernatant) at the top. The supernatant was observed for free drug at »max 205nm using Shimadzu-1700 UV spectrophotometer[12,13]. The % encapsulation efficiency (E) was calculated using the following formula:-

$$\% E = (TD - UD) / TD \times 100$$

Where, TD = total drug concentration and UD = concentration of unencapsulated drug.

In-vitro drug permeation studies: The in-vitro drug permeation studies were performed using Franz diffusion cell. The drug formulation was placed in the donor compartment while the receptor compartment contained 65 ml, 0.9% NaCl at pH 7.4. The dialysis membrane was so placed that it just touched the solvent portion. As the dialysis membrane correlates with that of the skin, it acts as a permeation barrier in the diffusion cell. Sodium lauryl sulphate (1%) was added to the receptor compartment to ensure sink conditions. Aliquots of 2.0 ml samples were withdrawn at 0,1,2,3,4,5,6,7 and 8 hr from the receptor compartment and the drug was assayed spectrophotometrically at »max 205 nm[14].

Storage stability studies: To observe the ability of the liposomal vesicles to retain drug, the liposomal suspension were sealed in ampules (10 ml capacity) and stored at different temperatures i.e. in refrigerator (2 - 8ÚC), room temperature, 37±2ÚC and 45±2ÚC for 4 weeks. Samples were withdrawn periodically and analysed for the drug content, in the manner desirable under drug entrapment studies.

Table 1

Effect of drug – lipid ratio &amount of cholesterol on % drug loading in the liposomal formulations

S No.	Formulation Code	Composition PC : CHol : Drug	% Drug Loading
1	L1	50:50:20	45.25 ± 2.49
2	L2	60:40:20	67.32 ± 1.42
3	L3	70:30:20	67.28 ± 3.24
4	L4	80:10:20	63.16 ± 5.74
5	L5	90:10:20	57.45 ± 2.88

* Values indicates mean ± SD (n=3)

Table 2
In-vitro permeation from different formulations of Aceclofenac

S. No	Aceclofenac Formulations	Mean Cumulative % drug permeated in 8 hrs
1	Aqueous Drug solution	31.24 ± 1.98
2	Drug in Carbopol Gel	28.16 ± 3.24
3	Liposomal Suspension	60.48 ± 4.21
4	Liposomal Gel	61.26 ± 2.38

* The experiment was done in triplicate (n=3)

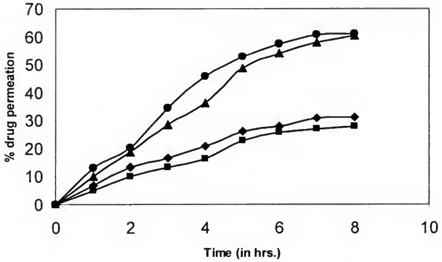


Figure 1. Percentage Drug permission of Aceclofenac

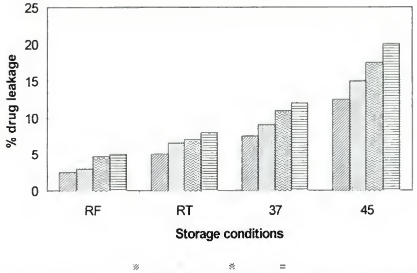


Figure 2. Extent of Drug leakage from L3 liposomes at different stroges temperatures

RESULTS AND DISCUSSION

Transmission electron microscopy: TEM photograph showed that the size of the optimized formulation was 882.245±0.01 nm. The increase in vesicle size may be because of the increase in the lipid mass ratio, which indicated that all the vesicles are multilamellar in nature.

Vesicle size analysis: The average vesicle size was found to be 6.64±1.52; 1.42±0.50; 3.72±0.74; 7.56±0.77 and 8.62±0.82 µm for formulations L1,L2,L3,L4 and L5, respectively. The experiment was performed in triplicate (n=3).

Vesicle count: Vesicle count was done by using hemocytometer, and was found to be (12.32±1.42; 15.21±2.24; 21.10±2.86; 28.18±1.91 and 16.34±2.88) X 1000/ mm^3 for the formulations L1,L2,L3,L4 and L5, respectively. The experiment was performed in triplicate (n=3).

Drug entrapment efficiency: On decreasing the amount of cholesterol in the formulation; the encapsulation efficiency was found to be increased. As the ratio of soya lecithin is increased, the drug loading increases until a definite limit for lipophilic drugs.

In-vitro drug permeation studies: The results of the in-vitro drug permeation studies conducted with different formulations of Aceclofenac are given in table 2. The amount of drug permeated in 8 hours from liposomal suspension and liposomal gel were 60.4% and 61.2%, respectively, whereas the drug permeated in case of aqueous solution and drug in carbopol gel was found to be only 31.2% and 28.1% respectively. (Figure - 1). The results show significant increase in permeation of Aceclofenac from the liposomal formulations.

Storage stability studies: The L3 liposomes were found to be reasonably stable in terms of fusion and/or vesicle rupture, over the studied storage period. They also retained their multilamellar nature and shape uniformity. The bar diagram (Figure - 2) depicts the percent drug leakage from the liposomes for 4 weeks, at different storage temperatures. Substantial loss of drug, i.e. 12 to 20% was noticed at elevated temperatures, viz. 37±2ÚC and 45±2ÚC, respectively. On the other hand, at lower temperatures, viz. RT and RF, the liposomes retained 92 to 95% of the incorporated drug, respectively. Loss of drug from the liposomes may be due to the effect of temperature on the gel to liquid transition of lipid bilayers and possible degradation of phospholipids, leading to leakage of drug from liposomes. It was observed that on decreasing the concentration of cholesterol; the drug release and stability behaviour of the formulation is increased. Incorporation of bioadhesive polymer carbopol 974P in the liposomes led to increase in the stability of the formulations. It was thus concluded that Aceclofenac has the potential to produce sustained effect for topical application as liposomal gel formulation.

REFERENCES

1. Pavelic Z, Basnet NS, Jalsenjak I. Liposomal gel with chloramphenicol: Characterisation and in vitro release. Acta Pharm. 2004; 54: 319-330.
2. Elzainy AAW, Gu X, Simons ER, Simons KJ. Cetrizine from topical phosphatidylcholine-hydrogenated liposomes. Immunol. 1999; 83: 445-448.
3. Schaeffer HE, Krohn DL. Liposomes in topical drug delivery. Invest.Ophthalmol.Vis.Sci. 1982; 22(2): 220-227.
4. Shende PK, Gaud RS. Simultaneous estimation of Aceclofenac and Paracetamol in combined dosage form by two wavelength spectrophotometry. The Pharma Review.2007
5. Nadkarni GD, Londhe VY, Nagarsenker S. Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. Int.J.Pharm. 1999; 190(1): 63-71.
6. Mutalik S. Preparations: In-vitro, preclinical and clinical evaluation of once daily sustained release tablets of Aceclofenac, Arch.Pharm.Res. 2007; 30(2): 222-234.
7. Patel VB, Misra AN, Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoyl peroxide for acne. Drug Dev.Ind.Pharm. 2000; 27(8):863-869.
8. Katare OP, Kumar R, Bhatia A. Tamoxifen in topical liposomes: development, characterization and in-vitro evaluation, J.Pharm. Sci. 2004; 7(2): 252-259.
9. Agarwal R, Katare OP. Miconazole nitrate-loaded topical liposomes. Pharm.Tech. 2002; 26: 48-60.
10. Paavola, Kilpelainen I, Yliruusi, Rosenberg P. Controlled release injectable liposomal gel of ibuprofen for epidural analgesia. Int.J.Pharm. 2000; 199(1): 85-93.
11. Ramana MV, Chaudhari AD, Himaja M, Satyanarayana D, Kamal D. An approach to minimize Pseudomembranous colitis caused by clindamycin through liposomal formulation, Indian J.Pharm.Sci. 2007; 69(3): 390-393.
12. Vyas SP, Singh R. Topical liposomes for localized and controlled drug delivery. J.Dermatol.Sci. 1996; 13: 107-11.
13. Qi XR, Liu MH, Liu HY, Maitani Y, Nagai T. Topical econazole delivery using liposomal gel. STP Pharma Sci. 2003; 13(4): 241-245.
14. GlavasDodov M, Goracinova K, Mladenovska, Kumbaradzi EF. Release profile of lidocaine HCl from topical liposomal gel formulation. 2002; 242(1-2): 381-384.

Instruction to Author

The Pharmacist is a bi-annual Journal, which publishes original research work that contributes significantly to further the scientific knowledge in Pharmaceutical Sciences (Pharmaceutical Technology, Pharmaceutics, Biopharmaceutics, Pharmacokinetics, Pharmaceutical/Medicinal Chemistry, Computational Chemistry and Molecular Drug Design, Pharmacognosy and Phytochemistry, Pharmacology, Pharmaceutical Analysis, Pharmacy Practice, Clinical and Hospital Pharmacy, Cell Biology, Genomics and Proteomics, Pharmacogenomics, Bioinformatics and Biotechnology of Pharmaceutical Interest). The Journal publishes original research work either as a Full Research Paper or as a Short Communication. Review Articles on a current topic in Pharmaceutical Sciences are also considered for publication by the Journal.

Type-written manuscripts prepared using MS Word should be submitted in triplicate to;

The Editor,
The Pharmacist
A-14, Siddharth Lake City
Anand Nagar,
Bhopal - 462021 (M.P.)
India

Alternatively, the manuscript can also be sent as a MS word document to **thepharmacist_ijp@rediffmail.com**. Manuscripts will be subjected to peer review process to determine their suitability for publication provided they fulfil the requirements of the journal as laid out in the instructions to authors. After the review, manuscripts will be returned for revision along with reviewer's and/or editor's comments. **One original copy of the final revised manuscript should be submitted for publication with in two months of receiving the reviewer's comments.** It is also desirable to submit the final revised manuscript on a CD prepared in MS Word Version 97-2002 or a higher version. Prepare the manuscript in Times New Roman font using a font size of 12. Title shall be in a font size 14. All section titles in the manuscript shall be in font size 12, bold face capitals. Subtitles in each section shall be in font size 12, bold face lower case followed by a colon. There shall not be any decorative borders anywhere in the text including the title page. The entire MS Word document with graphs and illustrations pasted in it shall not exceed 1.2 Mb.

Submission of a manuscript to The Pharmacist for publication implies that the same work has not been either published or under consideration for publication in another Journal. Authors, in their cover note to the Editor, have to clearly mention whether the manuscript shall be considered as a Research Paper, Short Communication or Review Article and also confirm that the manuscript has not been submitted to any other Journal for publication. **Authors publishing results from in vivo experiments involving animals or humans should state whether due permission for conduction of these experiments was obtained, from the relevant ethics committees, in the Materials and Methods section. In addition, authors wishing to publish research work involving human studies should also send a notary verified letter of approval from the Ethics Committee or the Institutional Review Board.**

PREPARATION OF MANUSCRIPTS:

Reserch Papers

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by International Committee of Medical Journal Editors (October 2001).

Authors are advised to go through the current set of Instructions to Authors and prepare their manuscripts accordingly. Information should be conveyed in simple language with the correct syntax. Care should be taken to avoid common errors like having abbreviations in the Title or Abstract, beginning a sentence with a numeral, adding "etc." after a few examples, dropping articles and using "&" instead of 'and' in the text. The usage of standard abbreviations and symbols is encouraged. Manuscripts that fail to conform to the requirements of the Journal, as specified under Instructions to Authors, will be rejected outright.

Manuscripts should be concisely written and conform to the following general requirements: Manuscripts should be typewritten in double-space in A4 sized sheets, only on one side, with a 2 cm margin on both sides. Not including illustrations, Research Papers, should not exceed 6-7 pages, Review Articles, 10-15 pages and Short Communications, 3-4 pages. Pages should be numbered consecutively, starting with the title page and the matter arranged in the following order: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Acknowledgements, References, Illustrations (Tables and Figures including chemistry schemes along with titles and legends) and Figure and Table titles and legends. Abstract should start on a separate page and each table or figure should be on separate sheets. The titles "Abstract" and "Introduction" need not be mentioned. All other section titles should be in capital letters while subtitles in each section shall be in bold face lower case followed by a colon.

Title Page – Title page should contain title of the paper in bold face, title case (font size 14), names of the authors in normal face, upper case (font size 12) followed by the address(es) in normal face lower case. The author to whom all correspondence be addressed should be denoted by an asterisk mark. The title should be as short as possible and precisely indicate the nature of the work in the communication. Names of the authors should appear as initials followed by surnames for men and one given-name followed by surname for women. Full names may be given in some instances to avoid confusion. Names should not be prefixed or suffixed by titles or degrees. Names should be followed by the complete postal address or addresses with pin code numbers of the place(s), where the research work has been carried out. At the bottom left corner of the title page, please mention "*For correspondence" and provide a functional e-mail address. Address of the corresponding author to whom all correspondence may be sent should be given only if it is different from the address already given under authors' names. Trivial sub-titles such as 'Title', 'Author', 'Address' or 'Place of Investigation' shall not be included in tthe title page. Title page should be aligned centre except for "*For correspondence".

Provide a running title or short title of not more than 50 characters

Abstract - Should start on a new page after the title page and should be typed in single-space to distinguish it from the Introduction. Abstracts should briefly reflect all aspects of the study, as most databases list mainly abstracts. Short Communications as well as Review Articles should have an Abstract.

Key-words: Provide four to ten appropriate key words after abstract preferably from McSH terms provided by National Library of Medicine

Introduction - Shall start immediately after the Abstract, as the next paragraph, but should be typed in double-space. The Introduction should lead the reader to the importance of the study; tie-up published literature with the aims of the study and clearly states the rationale behind the investigation.

Materials and Methods - Shall start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned. The main methods used shall be briefly described, citing references. Trivial details may be avoided. New methods or substantially modified methods may be described in sufficient detail. The statistical method and the level of significance chosen shall be clearly stated. IJPS prefers to publish work that has been subjected to an appropriate statistical test at one level of significance.

Results - All findings presented in tabular or graphical form shall be described in this section. The data should be statistically analyzed and the level of significance stated. Data that is not statistically significant need only to be mentioned in the text - no illustration is necessary. All Tables and figures must have a title or caption and a legend to make them self-explanatory. Results section shall start after materials and methods section on the same page.

Discussion - This section should follow results, deal with the interpretation of results, convey how they help increase current understanding of the problem and should be logical. Unsupported hypothesis should be avoided. The Discussion should state the possibilities the results uncover, that need to be further explored. There is no need to include another title such as "Conclusions" at the end of Discussion. Results and discussion of results can also be combined under one section, Results and Discussion.

Acknowledgements - Should be given after the text and not in the form of foot-notes.

References – References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. Use the style of the examples below, which are based on the formats used by the NLM in Index Medicus. The titles of journals should be abbreviated according to the style used in Index Medicus. Use complete name of the journal for non-indexed journals. Avoid using abstracts as references. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text. For scientific articles, contributors should obtain written permission and confirmation of accuracy from the source of a personal communication. The commonly cited types of references are shown here, for other types of references such as electronic media, newspaper items, etc. please refer to ICMJE Guidelines (http://www.icmje.org).
Articles in Journals :

1. Gawade SP, Anita Sankar. Pharmaco-photodynamics of photo-oxidised Russell's Viper venom product generated using UV radiation in the presence of methylene blue. Indian J. Pharm. Educ. Res. 2007; 41(2): 121-128.
   List the first six contributors followed by et al.
2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
3. Issue with supplement: Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996; 23(1, Suppl 2):89-97.

Books and Other Monographs

4 Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

5. Editor(s), compiler(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

6. Chapter in a book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Illustrations

Tables - Should be typed on separate sheets of paper and should not preferably contain any molecular structures. Only MS word table format should be used for preparing tables. Tables should show lines separating columns but not those separating rows except for the top row that shows column captions. **Tables should be numbered consecutively in Arabic numerals and bear a brief title in capital letters** normal face. Units of measurement should be abbreviated and placed below the column headings. Column headings or captions hall be in bold face. It is essential that all tables have legends, which explain the contents of the table. Tables should not be very large that they run more than one A4 sized page. Tables should not be prepared in the landscape format, i. e. tables that are prepared widthwise on the paper.

Figures - Should be on separate pages but not inserted with in the text. **Figures should be numbered consecutively in Arabic numerals and bear a brief title in lower case bold face letters** below the figure. Graphs and bar graphs should preferably be prepared using Microsoft Excel and submitted as Excel graph pasted in Word. These graphs and illustrations should be drawn to approximately twice the printed size to obtain satisfactory reproduction. As far as possible, please avoid diagrams made with India ink on white drawing paper, cellophane sheet or tracing paper with hand written captions or titles. Photographs should be on glossy paper. Photographs should bear the names of the authors and the title of the paper on the back, lightly in pencil. Alternatively photographs and photomicrographs can be submitted as jpeg images. Figure and Table titles and legends should be typed on a separate page with numerals corresponding to the illustrations. Keys to symbols, abbreviations, arrows, numbers or letters used in the illustrations should not be written on the illustration itself but should be clearly explained in the legend. Avoid inserting a box with key to symbols, in the figure or below the figure. In case of photomicrographs, magnification should be mentioned either directly on them or in the legend. Symbols, arrows or letters used in photomicrographs should contrast with the background. Method of staining should also be mentioned in the legend.

General Guidelines for units and symbols - The use of the International System of Units (SI) is recommended.

Physical quantity	Base unit	SI Symbol	Specification	Example	Correct style
Length	meter	m			
Mass	gram	g	Use lowercase for	kilogram	kg
kilogram		kg	symbols or		
Time	second	s	abbreviations,		
	minute	m	Symbols are not	meter	m
	hour	h	followed by a		
	day	d	period, exception		
	week	w	end of sentence		
	month	m	Do not pluralize symbols	kilogram	kg
	year	y	When numbers are	100 meters	100 m
Amount of substance	mole	mol	printed symbols are		
Area	square meter	m^2	preferred		
Volume	cubic meter	m^3	Space between number	2mol	2 mol
	liter	l	and symbol	10mg	10 mg
	milliliter	ml	Place a zero before		0.01
	microliter	µl	a decimal		
			Decimal numbers are		0.75
			preferable to fractions		1 500 000
			Space used to separate		
			long number		
			exception four-digit numbers		1000

Chemical terminology - The chemical nomenclature used must be in accordance with that used in the Chemical Abstracts.

Symbols and abbreviations - Unless specified otherwise, all temperatures are understood to be in degrees centigrade and need not be followed by the letter 'C'. Abbreviations should be those well known in scientific literature. In vitro, in vivo, in situ, ex vivo, ad libitum, et al. and so on are two words each and should be written in italics. None of the above is a hyphenated word. All foreign language (other than English) names and words shall be in italics as a general rule. Words such as carrageenan-induced inflammation, paracetamol-induced hepatotoxicity, isoproterenol-induced myocardial necrosis, dose-dependent manner are all hyphenated.

Biological nomenclature - Names of plants, animals and bacteria should be in italics.

Enzyme nomenclature - The trivial names recommended by the IUPAC-IUB Commission should be used. When the enzyme is the main subject of a paper, its code number and systematic name should be stated at its first citation in the paper.

Spelling - These should be as in the Concise Oxford Dictionary of Current English.

SHORT COMMNUNICATIONS

The journal publishes exciting findings, preliminary data or studies that did not yield enough information to make a full paper as short communications. These have the same format requirements as full papers but are only up to 5 pages in length. Short Communications should not have subtitles such as Introduction, Materials and Methods, Results and Discussion - all these have to be merged into the running text. Short Communications preferably should have only 3-4 illustrations.

REVIEW ARTICLES

Should be about 15 pages long, contain up-to-date information, comprehensively cover relevant literature and preferably be written by scientists who have in-depth knowledge on the topic. All format requirements are same as those applicable to full papers. Review articles need not be divided into sections such as materials and Methods and Results and Discussion, but should definitely have an Abstract and Introduction, if necessary.

Author pay charges: Because of high cost of publishing article in the journal payment for publication is mandatory and subjected to changes with out notice. Currents charges are Rs. 600/- for the first three pages (inclusive of charts table and references) subsequent pages will be charged at the rate of Rs 200/- per page. Co authors above two are requested to tale an indivudial annual subscription at Rs.600/-

Demand draft should be made in favor of **The Pharmacist** from any nationalized bank playable at Bhopal (M.P).

ABBREVIATIONS OF JOURNAL NAMES

American Journal of Cardiology, The- **(Amer J Cardiol)**
American Journal of Hospital Pharmacy- **(Amer J Hosp Pharm)**
American Journal of Medicine, The- **(Amer J Med)**
American Journal of Medical Sciences, The- **(Amer J Med Sei)**
American Pharmacy- **(Amer Pharm)**
American Journal of Pharmacy- **(Amer J Pharm)**
American Journal of Physiology, The- **(Amer J Physiol)**
Analytical Chemistry- **(Anal Chem)**
British Journal of Experimental Pathology, The- **(Brit J Exp Pathol)**British Journal of Pharmacology and Chemotherapy- **(Brit J Pharmaeol)**
British Heart Journal- **(Brit Heart J)**
Canadian Journal of Pharmaceutical Sciences- **(Can J Pharm Sei)**
New England Journal of Medicine- **(N Engl J Med)**
Pharmaceutical Journal, **The (Pharm J)**
Pharmacological Research Communications- **(Pharmaeol Res Commun)**
Science- **(Seience)**Canadian Medical Association Journal- **(Can Med Assoe J)**
Cardiovascular Research- **(Cardiovase Res)**
Chemical and Engineering News- **(Chem Eng News)**
Chemist and Druggist, The- (b)
Clinical Pharmacokinetics- **(Clin Pharmacokinet)**
Current Sciences (India)- **(Current Sei (India)**
Drug Development and Industrial Pharmacy- **(Drug Develop Ind Pharm)**
Helvitica Chimica Acta- **(Helv Chim Aeta)**
Indian Journal of Medical Sciences- **(Indian J Med Sci)**
Indian Journal of Pharmaceutical Sciences- **(Indian J Pharm Sei)**
Journal of the American Chemical Society, The- (J **Amer Chem Soc)**
Journal of Biological Chemistry- **(J Biol Chem)**
Journal of Controlled Release- **(J Control Release)**
Journal of Medicinal Chemistry- **(J Med Chem)**
Journal of Organic Chemistry, The- **(J Org Chem)**
Journal of Pharmacology and Experimental Therapeutics- (**J Pharmaeol Exp Ther)**
Journal of Pharmacy and Pharmacology- **(J Pharm Pharmaeol)**
Lancet, The- **(Laneet)**
Nature- **(Nature)**

A Milestone in scientific research

Major vision of MRL is to co-ordinate and conduct research in the field of Pharmaceutical sciences and life sciences to develop scientific strategies and awareness among the young researchers. The laboratory is involved in the research and disseminates scientific information through publications, meetings and training courses. MRL focus on various research areas in the field of Lifesciences and Pharmaceutical sciences.

Research Activities

Micro Biology, Bio technologies, Cell culture studies, Animal Experimentation, Formulation & Development, Pharmacology, Pharmacognosy, Synthetic Chemistry & Extraction.

Certificate training CoursesProject & Dissertation training

Six months & Three months Project work or dissertation training will be provided throughout the year for all Lifesciences and Pharmaceutical sciences Postgraduate students (M.Sc & M.Pharm) on the basis of their area of interest.

Short term training & Long term training courses

Short term training (Duration - 15 days) & Long term training (Duration - 30 days) courses will be provided for all Lifesciences and Pharmaceutical sciences - Undergraduate & Postgraduate students (B.Sc, M.Sc, B.Pharm & M.Pharm).

Contract Research

MRL invites contract based research with the mutual understanding of the institute and investigators. On payable system, the requested research work will be performed and provided to the researchers who lack their research facilities in their own institute.

meru
research
institute

Pharmacy & Life science Professionals Training Institute
B-22, Siddharth Lake City, Anand Nagar, Bhopal-21
Contact: +91-9229999250

THE PHARMACIST

Volume 2, Number 2, 2007

RESEARCH PAPERS

Study of Hepatoprotective Activity of *Portulaca oleracea* L. on Rifampicin Induced Hepatotoxicity in Rat 1-5
A.S. Kulkarni, B.D. Siraskar, A.D. Deshpande, A.V. Kulkarni, S.M. Dhonde, and Bingi

***In vitro* Screening of *Ziziphus jujuba* for their Antioxidant and Anti-adipogenic activity** 7-10
A.R.Srividya P. Hari Balaji, H. Raghu and P. Vijayan

Evaluation of Anti-Inflammatory Potential of *Pisonia grandis* Leaf Extract in Rats. 11-12
N.Balakrishnan, A.B.Panda, Sanjiv Singh, Navin R Raj, C.S.SenthilKumar and A.Sathish

Effect of Rosiglitazone on Fluid and Electrolytes Transport in Jejunum and Colon of Rat *in vivo:* - A Possible Mechanism of Action 13-14
Sanjiv Singh, Jitendra Malik and B.M. Patil

Antimicrobial Evaluation of the Leaves of *Ervatamia coronaria* 15-16
Navin R. Raj, Chanchal N. Raj, Pushpendra Kannojia, N. Balakrishnan and A. B. Panda

Comparative Study of Polyherbal Formulation Cebrotone and Neurocetam for Neurocognition Enhancement 17-19
V. Khare, Rewa Verma, P. Kannojia, B.J. Mishra and Richa Mishra.

Formulation and Evaluation of Transdermal Patch of Colchicine with Release Modifiers 21-23
Kuldeep Ganju, Avinash Kondalkar and A.K.Pathak

Preliminary Pharmacognostic Evalutions and Phytochemical Studies on Leaf of *Chenopodium album* (Bathua Sag) 25-27
M.S.Pande and A.K. Pathak

After Use Wastage of Semisolid Tube Packaging: A Study 29-31
Archana Singh, Rajesh S Jadon, D.C. Goupale and S. Nayak

Estimation of Levocetrizine UV/Vis Spectroscopic Method in Tablet Dosage From 33-35
Atul Tripathi, Prashant Ghode, Hemant Dhongade and S.Nayak

Comparative *in vitro* Evaluation of Government Hospital Supplied and Commercial Brands of Paracetamol Tablets 37-39
A. Chourasia, B. J. Mishra, Richa Mishra and P. Kannojia

Topical Liposomal Gel with Aceclofenac: Characterization and *in vitro* Evaluation 41-44
Akanksha Garud, Navneet Garud, Vivek Tomar and Mayank Maheshwari

Copyrighted material